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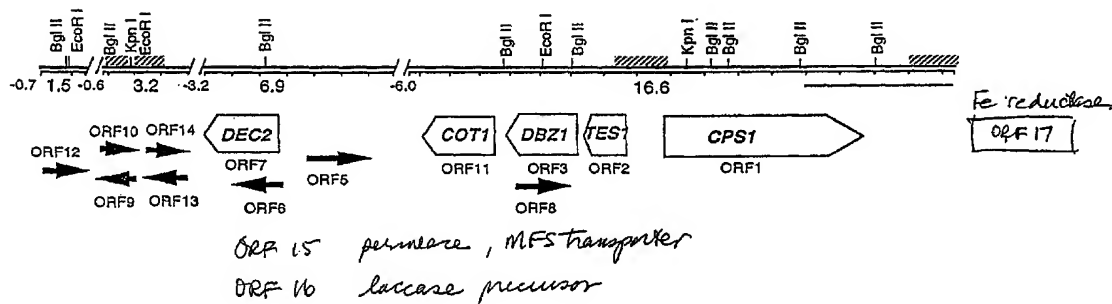
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(54) Title: FUNGAL GENE CLUSTER ASSOCIATED WITH PATHOGENESIS



(57) Abstract: Methods to identify orthologs of fungal CPS1 genes as well as fungal iron reductase and permease/and or MFS transporter genes, and uses thereof are provided.

FUNGAL GENE CLUSTER ASSOCIATED WITH PATHOGENESIS

Cross-Reference to Related Applications

5 This application claims the benefit of the filing date of U.S. application Serial No. 60/252,649, filed on November 22, 2000, and U.S. application Serial No. 60/252,732, filed November 22, 2000, under 35 U.S.C. § 119(e), the disclosures of which are incorporated by reference herein.

Statement of Government Rights

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The present invention was made with support from the United States Government (grant No. 96-35303-3198 from the USDA/NRI). The United States Government may have certain rights in the invention.

Field of the Invention

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The present invention relates to DNA molecules comprising fungal, e.g., *Cochliobolus heterostrophus*, genes from a peptide synthetase gene cluster, e.g., an iron reductase and/or a permease or major facilitator superfamily transporter, and uses thereof.

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Background of the Invention

There are approximately 30 species included in the genus *Cochliobolus*, nearly all of which are pathogens of wild grasses or cereals (Yoder et al., In: The Mycota Vol. 5: Plant Relationships, Part A, Berlin: Springer-Verlag, Carroll, eds., pp. 145-166 (1997)). *Cochliobolus heterostrophus* represents the most
25 widely distributed species in the genus and can be found in many tropical and subtropical areas in the world. As a natural pathogen of corn, *C. heterostrophus* causes a disease frequently called leaf spot of maize in the old literature (Drechsler, J. Agr. Res., 31:701 (1925); Drechsler, Phytopathol., 24:953 (1934);
30 Yu, "Studies on *Helminthosporium maydis*," 36:327 (1952)). In the United States, *C. heterostrophus* is usually found in the warmer southern states, thus, the

disease is commonly known as Southern Corn Leaf Blight (Hooker, Ann. Rev. Phytopathol., 12:167 (1974)). For many years, Southern Corn Leaf Blight was only known as an endemic disease and was not considered to be major economic importance in the United States. But in 1970, it suddenly broke into a severe epidemic that destroyed 15% of the U.S. corn crop and caused losses estimated at more than \$1 billion. This serious damage made Southern Corn Leaf Blight one of the most widely known crop diseases in the U.S.

Prior to the outbreak of the disease, only one race of *C. heterostrophus* (race O) was known in the field. In late 1969 when the disease became an epidemic, a new race of the fungus was identified from infected corn leaves collected in severely diseased areas. It was soon designated as race T because of its high virulence on T-cytoplasm corn and the ability to produce a phytotoxin called T-toxin, which specifically affects T-corn. In contrast, race O does not produce T-toxin and is mildly virulent on both T-cytoplasm and N-cytoplasm (normal cytoplasm) corn (Hooker et al., Plant Dis. Repr., 54:1109 (1970); Scheifele, "Cytoplasmically Inherited Susceptibility to Diseases Related to Cytoplasmically Controlled Pollen Sterility in Maize," 25:110 (1970); Smith et al., Plant Dis. Rep., 54:819 (1970); Yoder et al., Phytopathology, 65:273 (1975); Yoder, In: Biochemistry and Cytology of Plant Parasite Interaction, New York, New York:Elsevier, Tomiyama, eds., pp. 16-24 (1976); Yoder, Ann. Rev. Phytopathol., 18:103 (1980)). T-cytoplasm stands for Texas male sterile cytoplasm, a unique cytoplasm with a trait for maternally inherited male sterility, characterized by the failure to produce pollen (Levings, Science, 250:942 (1990)). T-cytoplasm corn was widely used for hybrid seed production and breeding to avoid hand or mechanical emasculation in the 1950s and the 1960s. It was the coexistence of large acreages of intensively planted T-cytoplasm corn and the sudden appearance of race T of *C. heterostrophus* that resulted in the epidemic of the disease in 1970. This discovery first opened the door to understanding pathogenesis by *C. heterostrophus*.

Early genetic analysis suggested that both T-toxin production and high virulence on T-cytoplasm corn are controlled by a single genetic locus defined as

Tox1 (Leach et al., Physiol. Plant Pathol., 21:327 (1982)). This was demonstrated by crosses between race T and race O in which only parental phenotypes segregated in a 1:1 ratio (Tox+:Tox-); all T-toxin producing progeny are highly virulent on T-cytoplasm corn while all T-toxin nonproducing progeny are weakly virulent (Yoder et al., 1975, *supra*; Leach et al., 1982, *supra*). Further investigation by comparison of electrophoretic karyotypes and chromosome-specific DNA hybridizations indicated that *Tox1* is tightly linked to a reciprocal translocation breakpoint and is associated with as much as a megabase of DNA (mostly highly repeated and A+T-rich) that is missing in race O (Bronson, Genome, 30:12 (1988); Tzeng et al., Genetics, 130:81 (1992); Chang et al., Genome, 39:549 (1996)). Surprisingly, recent analysis of several *Tox* mutants revealed that *Tox1* is not a single locus but rather two loci, each on a different translocated chromosome (Yoder et al., In Host-Specific Toxin: Biosynthesis, Receptor and Molecular Biology, Tottori, Japan: Faculty of Agriculture, Tottori Univ., Kohmoto, eds., pp. 23-32 (1994); Turgeon et al., Can. J. Bot., 73:S1071 (1995)). These two *Tox1* loci have been designated *Tox1A* and *Tox1B* (Yoder et al., 1997, *supra*). Two genes *PKS1* and *DECI* have been cloned from the two loci respectively; both are required for biosynthesis of T-toxin and are found only in race T isolates of *C. heterostrophus* (Yang, "The Molecular Genetics of T-Toxin Biosynthesis by *Cochliobolus heterostrophus*," Ph.D. Thesis, Cornell University (1995); Yang et al., Plant Cell, 8:2139 (1996); Rose et al., 8th Int. Symp. Mol. Plant-Microbe Int., Knoxville, p. J-49 (1996)).

Genetic analysis also suggested that T-toxin is required by *C. heterostrophus* for its high virulence on T-cytoplasm corn. This hypothesis was first tested by the generation of induced T-toxin deficient mutants using different mutagenesis procedures. All mutants with a tight *Tox*⁻ phenotype cause disease symptoms that are indistinguishable from those caused by race O when tested on both T and N-cytoplasm corn, suggesting that T-toxin is indeed a virulence factor (Yang et al., 1992; Lu et al., Proc. Natl. Acad. Sci. USA, 91:12649 (1994); Rose et al. (1996), *supra*). This conclusion was firmly supported by the site-specific disruption of the *PKS1* or *DECI* in the wild type race T genome;

disruptants lost the ability to produce T-toxins and caused race O type symptoms on both T-corn and N-corn (Yang et al., 1996, *supra*; Rose et al., 1996, *supra*). These experiments have given a very clear resolution for the role of T-toxin in pathogenesis. They also implied that pathogenesis by *C. heterostrophus* must involve additional pathogenicity factors because race O which does not produce T-toxin and race T-derived *Tox⁻* mutants are effective pathogens on corn.

A number of fungal molecules have been identified as general pathogenicity or virulence factors in several plant-pathogenic fungi (Yoder et al., *J. Genet.*, 75:425 (1996)). These include potential penetration factors such as melanin (Guillen et al., *Fungal Genet. Newsl.*, 41:41 (1994)), cutinase (Oeser et al., *Mol. Plant-Microbe Int.*, 7:282 (1994)) and polygalacturonase and xylanase (Lyngholm et al., *Fungal Genet. Newsl.*, 42:46 (1995)) or possible mechanisms involved in colonization such as phytotoxin detoxification (Schafer et al., *Science*, 246:247 (1989)) or components of signal transduction pathways. Although *C. heterostrophus* is known to produce a nonhost specific toxin called ophiobolin (or cochliobolin), a C₂₅ sesterterpenoid compound, which is toxic to many organisms, including plants, bacteria, fungi and nematodes, there is no evidence that ophiobolins are involved in pathogenesis by *C. heterostrophus* or other phytopathogenic fungi. No other pathogenesis-related toxins have been isolated from *C. heterostrophus* so far, but studies on closely related *Cochliobolus* species and other phytopathogenic fungi suggest that pathogenesis by this group of fungi also involves peptide toxins.

Four peptide phytotoxins (victorin, HC-toxin, AM-toxin, and enniatins) have been characterized as pathogenicity or virulence factors. They are all small cyclic peptides (4-6 residues), containing unusual amino acids or hydroxy acids, and they can be either host specific or non-host specific in terms of plant toxicity. A number of peptide phytotoxins are believed to be synthesized nonribosomally. Early in the 1960s, several biochemists working on the bacterial peptide antibiotics gramicidin and tyrocidine found that these polypeptides can be synthesized in RNAase-treated particle-free extracts of *Bacillus brevis* that are known to produce the same antibiotics; adding protein-

synthesis inhibitors to the extracts does not affect this process. This indicated the existence of a peptide biosynthetic system in which ribosomes and mRNAs are not needed. Further studies revealed that in this system, peptides are synthesized on a protein-template and this template itself is a multifunctional enzyme or a complex of several such enzymes, collectively called peptide synthetases, catalyzing the biosynthetic process (Laland et al., Essays in Biochemistry, 7:31 (1973); Lipmann, Adv. Microbiol. Physiol., 21:277 (1980)).

Peptide synthetases can catalyze biosynthesis of a variety of peptides. In terms of bioactivity, they can be antibiotics, enzyme inhibitors, plant or animal toxins and immunosuppressants (Stachelhaus et al., Journal of Biological Chemistry, 270:6163 (1995)). In terms of chemical structure, they can be either linear (i.e., ACV, the penicillin precursor and gramicidin) or cyclic (most are). The latter can be further classified into three subgroups: 1) The “standard” cyclic peptides (i.e., gramicidin S, tyrocidine, HC-toxin and cyclosporin); 2) cyclic lactones (i.e., destruxin); and 3) cyclic depsipeptides (i.e., beauvericin and enniatin). There have been over 300 different carboxy compounds that can be activated by peptide synthetases.

Although the first peptide synthetase, Gramicidin S synthetase, was purified and used for the cell-free synthesis of the peptide early in the 1960s (Tomino et al., Biochem, 6:2552 (1967)), the first bacterial peptide synthetase gene, *tycA*, which encodes the tyrocidine synthetase 1 in *B. brevis*, was not cloned until almost twenty years later (Marahiel et al., Mol. Gen. Genet., 201:1986 (1985)). Since then, more than twenty peptide synthetase genes have been reported for both bacteria and filamentous fungi, but only fourteen have complete nucleotide sequences published. All are larger than 3.3 kb and range between 3.3-19.5 kb for bacterial genes and 9.4-45.8 kb for fungal ones. Interestingly, all fungal peptide synthetase genes reported lack introns, even the cyclosporin A synthetase gene *simA*, which has a 45.8 kb of open reading frame (the largest genomic ORF so far recorded). Although biosynthesis of bacterial peptides differs from that of fungal ones in terms of the number of

multifunctional enzymes involved, the genes encoding these enzymes are similar to each other in both function and structure.

Comparison of nucleotide sequences reveals one or more highly conserved regions at certain positions in each peptide synthetase gene. These regions formerly called "amino acid activating domains" (Stachelhaus et al., 1995, *supra*), now called "amino acid activating modules" (Marahiel, Chem. Biol., 4:561 (1997)) consist of a set of domains (formerly called "modules") believed to have specific functions such as recognition, activation and thioesterification of individual constituent amino or hydroxy acids, and in some cases methylation and racemation for modification of certain residues before incorporation into the peptide chain (Stachelhaus et al., 1995, *supra*). The most convincing evidence supporting this assignment is that in most cases, the number of conserved functional units in each gene or gene cluster is equal to the number of amino acids in the respective peptide. This one-for-one match is very clear between three of four fungal peptides and their biosynthetic genes. The total number of modules in three of four bacterial gene clusters also matches the number of amino acids in the respective peptides.

Sequence alignment of amino acid-activating modules reveals strictly conserved sequence motifs that contain active residues for module functions. These motifs are called "core sequences" (Marahiel, FEBS Lett., 307:40 (1992)). A minimal amino acid-activating module must contain six core sequences, whose functions (except for core 1) have been proposed based on mutational analysis of several peptide synthetases. Core sequences 1-5 are grouped into an amino acid adenylation domain and core 6 is a thioester formation domain (Figure 1A). All bacterial peptide synthetase genes contain "type I modules," the minimal amino acid activating modules which were previously called "type I domains" (Stachelhaus et al., 1995, *supra*). Two fungal genes, *acvA* and *HTS1* also have this modular structure. In addition to the type I module, two fungal genes, *esynI* and *simA*, contain type II modules, in which an insertion (about 400 amino acids) is found between cores 5 and 6 of a normal type I module. This region contains a motif (VLE/DXGXGXG; SEQ ID NO:1), highly conserved in

S-adenosyl-methionine (SAM)-dependent methyltransferases, hence, it is referred to as a N-methylation domain (Figure 1A). Additional evidence for methyltransferase activity of this module is that the number and position of type II modules in *esyn1*, and *simA* exactly match that of N-methylated amino acids in ennatin and cyclosporin sequences (Figure 1B).

Although the modular structure described above is highly conserved among most peptide synthetase genes, some variations have been found in the latest cloned peptide synthetase gene *safB*, which is the first gene in the saframycin Mx1 synthetase gene cluster (Pospiech et al., Microbiology, 141:1793 (1995)). *safB* contains two type I amino acid activating modules. One module has all six highly conserved core sequences, but another, believed to activate alanine (the first amino acid in the linear tetrapeptide precursor of saframycin Mx1), lacks core 5 and has a weakly conserved core 1 (Pospiech et al., Microbiology, 142:741 (1996)) (Figure 1A). This suggests that some of the motifs in the amino acid adenylation domain are dispensable or not critical for domain function. It also raises the possibility that other variations might be found in yet unknown peptide synthetase genes.

Although *C. heterostrophus* has been a model eukaryotic plant pathogen since the 1970s, most molecular genetic analyses conducted in this system have focused on production of the polyketide T-toxin by race T isolates of the fungus. Solid evidence now indicates that T-toxin is a host-specific virulence factor in Southern Core Leaf Blight (Yoder et al., J. Genet., 75:425 (1996); Yoder et al., 1997). It is clear, however, that *C. heterostrophus* needs additional factors, presumably general factors for pathogenesis to corn plants, since race O, which does not produce T-toxin, can be an effective corn pathogen. Attempts to identify additional general factors required by *C. heterostrophus* for pathogenesis have been unsuccessful.

Thus, what is needed is the isolation and characterization of additional fungal genes that control the biosynthesis of novel fungal molecules associated with pathogenesis, i.e., genes which are potential targets for the design of

products that might interfere with the infection process, and vertebrate fungal orthologs of fungal peptide synthetase genes.

Summary of the Invention

5 The invention generally relates to an isolated nucleic acid molecule (polynucleotide), e.g., DNA or RNA, comprising a nucleic acid segment which encodes a gene product related to pathogenesis. In one embodiment of the invention, fungal genes which are related to pathogenesis are identified. An advantage of the present invention is that the genes described herein provide the

10 basis to identify a novel fungicidal or mycocidal mode of action which permits rapid discovery of novel inhibitors of gene products that are useful as fungicides or mycocides. In addition, the invention provides isolated genes or gene products from fungi for assay development for inhibitory compounds with fungicidal or mycocidal activity, as agents which inhibit the function or reduce or

15 suppress the activity of those gene products in fungi are likely to have detrimental effects on fungi, and are good fungicide or mycocide candidates. The present invention therefore also provides methods of using a polypeptide encoded by one or more of the genes of the invention or a cell expressing such a

20 polypeptide to identify inhibitors of the polypeptide, which can then be used as fungicides to suppress the growth of pathogenic fungi. Pathogenic fungi are defined as those capable of colonizing a host and causing disease. Examples of fungal pathogens include plant pathogens such as *Septoria trici*, *Ashbya gossypii*, *Stagenospora nodorum*, *Botryus cinera*, *Fusarium graminearum*, *Magnaporthe grisea*, *Cochliobolus heterostrophus*, *Colleototrichum*, *Ustilago maydis*,

25 *Erisyphe graminis*, plant pathogenic oomycetes such as *Pythium ultimum* and *Phytophthora infestans*, as well as dimorphic fungal pathogens including *Blastomyces*, e.g., *B. dermatitidis*, *Coccidioides*, *Histoplasma*, e.g., *H. capsulatum*, or *Paracoccidioides*, e.g., *P. brasiliensis*, *Loboa*, *Malassezia*, *Rhodotorrula*, *Blastoschizomyces*, *Trichosporon*, *Saccharomyces*, *Cryptococcus*

30 including *Cryptococcus neoformans*, as well as human pathogens such as *Candida albicans*, and other pathogenic *Candida*, e.g., *C. tropicalis*, *C.*

parapsolosis and *C. guettermondii*, *Coccidioides imitis*, and *Aspergillus fumigatus*, *Sporothrix schenckii*, pathogenic members of the Genera *Epidermophyton*, *Microsporum* and *Trichophyton*, *Cladosporium* (*Xylohypha*) *trichoides*, *Cladosporium bantianum*, *Penicillium marneffii*, *Exophiala* (5 *Wangiella*) *dermatitidis*, *Fonsecaea pedrosoi* and *Dactylaria gallopava* (*Ochroconis gallopavum*), and including mycogens. Preferred fungi for use with the agent identified by the method of the invention are *Ascomycota*.

In one embodiment of the invention, the invention relates to an isolated polynucleotide comprising a nucleic acid segment encoding an ortholog of a
 10 plant fungal CPS1, e.g., SEQ ID NO:3 from *Cochliobolus* which is a CoA ligase, or a nucleic acid segment encoding a gene product that modulates fungal iron metabolism, uptake, absorption of inorganic or organic ferric salts, e.g., a fungal iron reductase, permease or MFS transporter, e.g., a siderophore transporter, which genes maybe associated with *CPS1* in a gene cluster. As described herein
 15 below, a gene from *Coccidioides imitis* and *Candida* that is related to the *CPS1* gene of *Cochliobolus* was identified, e.g., a nucleic acid sequence comprising an open reading frame comprising SEQ ID NO:46 which encodes SEQ ID NO:47 or the complement thereof. The *CPS1* gene in *Cochliobolus* is present in a cluster of closely linked open reading frames, a cluster which is associated with
 20 virulence and/or pathogenicity, wherein CPS1 is representative of a novel class of adenylation domain-containing enzymes related to but distinct from nonribosomal protein synthetases (NRPSs). Thus, at least one of the genes in the cluster may control biosynthesis of a secondary metabolite (small molecule) that is required for or associated with fungal virulence and/or pathogenesis.
 25 Similarly, orthologs of the described *Cochliobolus* gene cluster, e.g., those in *Coccidioides* or *Candida*, may encode gene products that are required for or associated with fungal virulence. As also described hereinbelow, a *Cochliobolus* iron reductase (SEQ ID NO:49 encoded by SEQ ID NO:48) and a permease and/or MFS transport protein gene (SEQ ID NO:55 encoding SEQ ID NO:56)
 30 were identified that are closely linked to a *CPS1* peptide synthetase gene, e.g., a DNA molecule comprising SEQ ID NO:2 (GenBank accession no. AF332878)

encoding SEQ ID NO:3 (GenBank accession no. AAG53991), which is part of a gene cluster associated with virulence and/or pathogenicity.

Thus, at least one of the genes in the cluster may control biosynthesis of at least one secondary metabolite or other small molecule that is required for or associated with fungal growth, virulence and/or pathogenesis. The fungal produced siderophore may sequester iron from the environment or host to aid in fungal growth. *Pseudomonas aeruginosa* produces pigments that are likely associated with virulence, e.g., pyocyanin. A derivative of pyocyanin, pyochelin, is a siderophore that is produced under low iron conditions to sequester iron from the environment for growth of the pathogen. The competition for iron may have a deleterious effect on the host. Similarly, the *Cochliobolus* iron reductase or permease/transporter or other gene products associated with iron metabolism may compete with the host for Fe and so contribute to the pathogenicity of the fungus. Similarly, orthologs of the described genes in the *Cochliobolus* gene cluster in other fungi which infect plants or those that infects vertebrate animals may encode gene products that are required for or associated with fungal virulence including iron metabolism genes, e.g., genes associated with secretion of a toxin or siderophore.

Preferably, the nucleic acid segment is obtained or isolatable from a fungal gene which encodes a polypeptide which is substantially similar, and preferably has at least 70%, e.g., 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, and even 90% or more, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, up to at least 99%, amino acid sequence identity to, a polypeptide encoded by a nucleic acid sequence comprising any one of SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55, or a fragment (portion) thereof which encodes a partial length polypeptide having substantially the same activity of the full length polypeptide. Preferably, the activity of the partial length polypeptide is at least 50%, generally at least 60%, ordinarily at least 70%, preferably at least 80%, more preferably at least 90% and more preferably still at least 95% the activity as the full-length

polypeptide. Preferred partial length polypeptides have substantially the same activity as the corresponding full-length polypeptide.

Further provided is an isolated polynucleotide comprising a nucleic acid segment which is substantially similar, and preferably has 70%, e.g., 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, and even 90% or more, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, up to at least 99%, nucleotide sequence identity to, a nucleic acid sequence comprising an open reading frame comprising any one of SEQ ID NO: 46, SEQ ID NO:48, or SEQ ID NO:55.

Another aspect of the present invention, as described below, relates to a method for identifying inhibitors of the gene products encoded by the polynucleotides of the invention, which involves contacting the gene product or cell expressing the polynucleotide with agents that are potential inhibitor compounds, and selecting compounds which decrease the activity of the gene product and/or inhibit cell growth. In another embodiment, the invention relates to a method of imparting disease resistance to a plant or other organism by overexpression the CPS1 ortholog of the invention in the plant or other organism.

The nucleic acid molecules of the invention are preferably obtained or isolatable from a gene from fungi that infect vertebrates, including but not limited to mammals, e.g., livestock such as bovine, ovine, porcine, equine and avians such as turkey and chickens and domestic pets including avians, feline and canine, and humans, which genes are related to pathogenesis. For example, preferred nucleic acid molecules of the invention are obtained or isolatable from *Ascomycetes* (ascomycetes), and the agents of the invention are useful to treat infections due *Ascomycota* infection, based on the discovery of CPS1, its orthologs and related genes in the cluster, in various ascomycetes human (and plant) pathogens as disclosed herein. Within pathogenic *Ascomycetes*, the following groups are of interest: *Agyriales*, *Arthoniales*, *Ascospaerales*, *Caliciales*, *Calosphaeriales*, *Capnodiales*, *Chaetothyriales* (black yeasts), *Cyttariales*, *Diaporthales*, *Dothideales*, *Elaphomycetales*, *Erysiphales* (powdery

- mildews), *Eurotiales* (green and blue mold), *Gyalectales*, *Halosphaeriales*, *Helotiales*, *Hypocreales*, *Laboulbeniales*, *Lecanorales*, *Lulworthiales*, *Melanommatales*, *Meliolales*, *Microascales*, *Myriangiales*, *Neoelectales*, *Onygenales*, *Ophiostomatales*, *Ostropales*, *Patellariales*, *Pertusariales*,
5 *Pezizales*, *Phyllachorales*, *Pleosporales*, *Protomycetales*, *Pyrenulales*, *Rhytismatales*, *Saccharomycetes*, *Schizosaccharomycetales*, *Sordariales*, *Taphrinales*, *Teloschistales*, *Thelebolaceae*, *Umbilicariales*, *Xylariales*, anamorphic *Ascomycota*, unclassified *Ascomycota*, and *Ascomycota incertae sedis*.
- 10 Regarding *Ascomycetes* animal pathogens, preferred are pathogenic *Onygenales*, more particularly the anamorphic *Onygenales*, which includes *coccidioides*, and the *Onygenaceae* and its group *Ajellomyces*, which includes *Histoplasma* such as *Histoplasma capsulatum*, and *Blastomycoides* such as *Blastomycoides dermatitidis*. Also preferred are pathogenic *Saccharomycetes*,
15 more preferably *Saccharomycetales*, and even more preferably anamorphic *Saccharomycetales*, which includes *Candida* species. Also preferred are *Chaetothyriales*, more preferably *Herpotrichiellaceae*, even more preferably anamorphic *Herpotrichiellaceae*, and even more preferably *Exophiala*, which include the human-pathogenic organisms *Exophiala dermatitidis* and *Exophiala*
20 *jeanselmei*. Also preferred are the *Onygenales*, more preferably *Arthrodermataceae*, more preferably anamorphic *Arthrodermataceae*, and even more preferably *Trichophyton*, which contain *Trichophyton rubrum*. Another preferred group is Fungi incertae sedis, more preferably *Pneumocystidaceae*, and even more preferably *Pneumocystis*, which includes the human pathogen
25 *Pneumocystis carinii*. Yet another preferred group is *Eurotiales*, more preferred *Trichocomaceae*, even more preferred anamorphic *Trichocomaceae*, and yet even more preferred is *Aspergillus* species, which contains *Aspergillus avenaceus* and *Aspergillus fumigatus*. Another preferred group are those pathogenic fungi in *Pleosporales*, more preferably *Pleosporaceae*, yet more
30 preferably anamorphic *Pleosporaceae*, and even more preferably *Alternaria* species, which includes airborne *Alternaria alternata*. Also preferred is

Ascomycota incertae sedis, more preferably *Mycosphaerellaceae*, particularly the anamorphic *Mycosphaerellaceae*, and more preferably the species *Cladosporium*, which includes airborne human pathogens. Also preferred are anamorphic *Ascomycota*, more preferably the species *Helminthosporium*.

- 5 Within Onygenales are preferably anamorphic *Onygenales*, and more preferably the *Paracoccidioides* species, which includes *Paracoccidioides brasiliensis*. Also preferred are *Microascales*, more preferably *Microascaceae*, and even more preferably *Pseudallescheria* species, which includes *Pseudallescheria boydii*. Also preferred are *Ophiostomatales*, more preferably *Ophiostomataceae*, yet
- 10 more preferably anamorphic *Ophiostomataceae*, and more preferably *Sporothrix* species, including *Sporothrix schenckii*.

- The term “substantially similar”, when used herein with respect to a polypeptide means a polypeptide corresponding to a reference polypeptide, wherein the polypeptide has substantially the same structure and function as the
- 15 reference polypeptide, e.g., where the only changes in amino acid sequences are those which do not affect the polypeptide function. When used for a polypeptide or an amino acid sequence, the percentage of identity between the substantially similar and the reference polypeptide or amino acid sequence is at least 70%, e.g., 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%,
- 20 84%, 85%, 86%, 87%, 88%, 89%, and even 90% or more, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, up to at least 99%, wherein the reference polypeptide comprises SEQ ID NO:47, SEQ ID NO:49 or SEQ ID NO:56. One indication that two polypeptides are substantially similar to each other is that an agent, e.g., an antibody, which specifically binds to one of the polypeptides,
- 25 specifically binds to the other.

- In its broadest sense, the term “substantially similar”, when used herein with respect to a nucleotide sequence or nucleic acid segment, means a nucleotide sequence or segment corresponding to a reference nucleotide sequence or nucleic acid segment, wherein the corresponding sequence encodes a
- 30 polypeptide having substantially the same structure and function as the polypeptide encoded by the reference nucleotide sequence or nucleic acid

segment. The term “substantially similar” is specifically intended to include nucleotide sequences wherein the sequence has been modified to optimize expression in particular cells. The percentage of identity between the substantially similar nucleotide sequence and the reference nucleotide sequence is at least 70%, e.g., 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, and even 90% or more, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, up to at least 99%, preferably wherein the reference sequence comprises SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55 or the complement thereof. Sequence comparisons may be carried out using a Smith-Waterman sequence alignment algorithm (see e.g., Waterman, Introduction to Computational Biology: Maps, sequences and genomes, Chapman & Hall, London (1995) or <http://www.htousc.edu/software/seqaln/index.html>). The local S program, version 1.16, is preferably used with following parameters: mat:1, mismatch penalty: 0.33, open-gap penalty:2, extended-gap penalty:2. Further, a nucleotide sequence that is “substantially similar” to a reference nucleotide sequence hybridizes to the reference nucleotide sequence under moderate, stringent, or very stringent, hybridization conditions, e.g., in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

Thus, the invention also includes recombinant nucleic acid molecules which have been modified so as to comprise codons other than those present in the unmodified sequence or have been modified by shuffling. The recombinant nucleic acid molecules of the invention include those in which the modified codons in the unmodified sequence, as well as those that specify different amino

acids, i.e., they encode a variant polypeptide having one or more amino acid substitutions relative to the polypeptide encoded by the unmodified sequence.

The invention further includes a nucleotide sequence which is complementary to one (hereinafter "test" sequence) which hybridizes under stringent conditions with the nucleic acid molecules of the invention as well as RNA which is encoded by the nucleic acid molecules of the invention as well as RNA which is encoded by the nucleic acid molecule. When the hybridization is performed under stringent conditions, either the test or nucleic acid molecule of the invention is preferably supported, e.g., on a membrane or DNA chip. Thus, either a denatured test or nucleic acid molecule of the invention is preferably first bound to a support and hybridization is effected for a specified period of time at a temperature of, e.g., between 55 and 70°C, in double strength citrate buffered saline (SC) containing 0.1% SDS followed by rinsing of the support at the same temperature but with a buffer having a reduced SC concentration. Depending upon the degree of stringency required such reduced concentration buffers are typically single strength SC containing 0.1% SDS, half strength SC containing 0.1% SDS and one-tenth strength SC containing 0.1% SDS.

Hence, the isolated nucleic acid molecules of the invention include orthologs of SEQ ID NO:46, SEQ ID NO:48 and SEQ ID NO:55, which includes orthologs of the polypeptides encoded therein. An ortholog is a gene from a different species that encodes a product having the same function as the product encoded by a gene from a reference organism. The encoded ortholog products likely have at least 68 to 70% (substantial) sequence identity to each other. Hence, one embodiment the invention includes an isolated polynucleotide comprising a nucleic acid segment encoding a polypeptide having at least 68 to 70% identity to a polypeptide encoded by SEQ ID NO:46, SEQ ID NO:48 or SEQ ID NO:55. Databases such as GenBank which can be accessed at <http://www.ncbi.nlm.nih.gov/>, may be employed to identify sequences related to those sequences. Alternatively, recombinant DNA techniques such as hybridization or PCR may be employed to identify sequences related to the sequences. Preferred orthologs include those from dimorphic fungal pathogens

including *Blastomyces*, e.g., *B. dermatitidis*, *Coccidioides*, *Histoplasma*, e.g., *H. capsulatum*, or *Paracoccidioides*, e.g., *P. brasiliensis*, *Loboa*, *Malassezia*, *Rhodotorrula*, *Blastoschizomyces*, *Trichosporon*, *Saccharomyces*, *Cryptococcus* including *Cryptococcus neoformans*, as well as human pathogens such as

5 *Candida albicans*, and other pathogenic *Candida*, e.g., *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii*, *Coccidioides immitis*, and *Aspergillus fumigatus*, *Sporothrix schenckii*, pathogenic members of the Genera *Epidermophyton*, *Microsporum* and *Trichophyton*, *Cladosporium* (*Xylohypha*) *trichoides*, *Cladosporium bantianum*, *Penicillium marneffii*, *Exophiala*

10 (*Wangiella*) *dermatitidis*, *Fonsecaea pedrosoi* and *Dactylaria gallopava* (*Ochroconis gallopavum*), as well as other mycogens.

The invention also provides anti-sense nucleic acid molecules corresponding to the sequences described herein. Also provided are expression cassettes, e.g., recombinant vectors, and host cells, comprising the nucleic acid

15 molecule of the invention in which the nucleic acid segment is in either sense or antisense orientation. Also provided is a microarray, comprising one or more of the nucleic acid molecules of the invention or a portion thereof.

Owing to the dramatically increased incidence of life-threatening opportunistic fungal infections it is now clear that diseases of fungal infection

20 are of major importance. The rise in cases has been particularly apparent in transplant recipients and others who are immunocompromised, especially AIDS patients. Besides more serious infections associated with these vulnerable groups, superficial infections such as ringworm and thrush have also become more prevalent. Despite recognizing the importance of fungi as a cause of

25 disease in man and animals, many of the more serious fungal infections remain difficult to diagnose and treat. Thus, there is a continuing need to identify agents to treat fungal infections of vertebrates, including immunocompromised vertebrates, and complications thereof, e.g., pneumonia, flu-like illness, erythema nodosum, erythema marginatum, arthritis, multiple thin-walled chronic cavities,

30 miliary disease, bone and joint infection, skin disease, soft tissue abscesses, meningitis, oropharyngitis, oesophagitis, vaginitis, onychomycosis,

endophthalmitis, paronychia, and inflammation of the urinary tract, kidney, liver, brain, gastrointestinal tract, and lung.

Thus, another aspect of the present invention relates to a method for identifying inhibitors of the fungal vertebrate CPS1 ortholog, or fungal iron reductase or permease/MFS transporter of the invention. For example, genes
5 encoding products that are associated with virulence, and agents that bind to or otherwise alter or modulate the activity of that gene product, preferably agents that inactivate or decrease (reduce or inhibit) the activity of the gene product, can be identified. The method comprises contacting the gene product(s) or cells
10 which express the gene product(s) with an agent and then determining or detecting whether the agent binds to, or decreases the activity of, the gene product(s). Such an agent modulates or alters a phenotype of the gene product or cell, e.g., pathogenicity of a cell which expresses the gene product. Modulation or alteration encompasses an increase as well as a decrease in an activity,
15 preferably the modification or alteration in the activity of the gene product or cell having the gene product contacted with the agent is at least 10%, or at least 50%, relative to the activity in an untreated control. In particular, the methods are useful to identify agents that inhibit, reduce or suppress the activity of the polypeptide, e.g., by at least 10%, preferably at least 50%, relative to the activity
20 in an untreated control. Thus, the invention also provides agents identified by the methods of the invention. Preferred agents bind to, more preferably inhibit, the activity of a polypeptide of the invention, e.g., one encoded by a dimorphic fungal pathogen such as one from *Blastomyces*, *Coccidioides*, *Histoplasma* or *Paracoccidioides*, and includes pathogenic *Candida*, e.g., *C. albicans*, *C. tropicalis*, *C. parapsolosis* and *C. guettermondii*. The methods may employ
25 screening agents on wild type fungi and/or recombinant fungi, e.g., fungi which overexpress the polypeptide of interest or do not express that polypeptide, e.g., as a result of expression of antisense sequences or a gene knock out. If the agent is one encoded by DNA, the expression of that DNA in an organism susceptible to
30 the pathogen, e.g., a plant, may provide tolerance or resistance to the organism to the pathogen, preferably by inhibiting or preventing pathogen infection.

Methods of the invention may include stably transforming a susceptible organism of cell with one or more sequences which confer tolerance or resistance operably linked to a promoter capable of driving expression of that nucleotide in the cells of the organism.

5 Other uses for the nucleic acid molecules or polypeptides of the invention, include the use of the polypeptide to raise either polyclonal antibodies or monoclonal antibodies, e.g., antibodies specific for the polypeptide, to detect antibodies in the serum of a vertebrate, or primers or probes specific for the nucleic acid molecules, which can be employed in diagnostic assays for the
10 presence of the pathogen or for therapeutic purposes, and host cells comprising the nucleic acid molecules, e.g., in antisense orientation, or having a deletion in at least a portion of at least one the genes corresponding to the nucleic acid molecules of the invention. Also, given that the gene may encode a peptide synthetase (Watanabe et al., Chem. Biol., 3, 463 (1996)) the gene product may be
15 useful in therapy, e.g., as an anti-cancer agent, an antibiotic, or as an immunosuppressant.

The agents identified by the methods of the invention may also be subjected to further assays to determine whether the agent is substantially non-toxic to a plant or vertebrate organism to be treated as well as the dose to be
20 administered to the vertebrate organism. For example, for *Coccidioides*, a murine model may be employed (see, Kirland et al., Infect. Immun., 40: 912 (1983)). This model may also be used for screening for an agent of the invention. Further, the agents identified by the methods of the invention, e.g., those which are non-toxic to a plant or vertebrate to be treated, are useful in
25 methods of preventing or treating a disease or disorder associated with fungal infection, including superficial, subcutaneous or systemic infections. The method comprises administering to a vertebrate or plant in need of such treatment, e.g., a vertebrate that is immunocompromised, an amount of an agent of the invention effective to inhibit or prevent fungal or mycogen infection or
30 growth. For example, humans and non-human animals including livestock and domestic pets may be treated with the agents of the invention, e.g., livestock

such as bovine, ovine, porcine, equine and avians such as turkey and chicken and domestic pets including avians, felines and canines. Preferably, the agents are administered topically to a mammal such as a human. Preferred plants include cereals, for example, corn, alfalfa, sunflower, rice, Brassica, canola, soybean, 5 barley, soybean, sugarbeet, cotton, safflower, peanut, sorghum, wheat millet, and tobacco.

Moreover, the agents of the invention may be used in conjunction with other therapeutic agents, e.g., fungicides, mycosides, and vaccines, including amphotericin B and azoles. In addition, the agents may be employed to treat 10 sources of fungal contamination, such as the soil or surface areas or materials on which fungi can survive and/or proliferate. Thus, the agents may be contacted with soil or other surfaces that come in contact with vertebrates. Although this contacting may not eliminate the fungus, it may reduce the risk of airborne dissemination of the fungus or its spores.

15 Also provided is a computer readable medium having stored thereon a nucleic acid sequence that is substantially similar to any one of SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55 or the complement thereof, and a computer system comprising a processor and data storage device wherein said data storage device has stored thereon a nucleic acid sequence that is substantially similar to 20 any one of SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55 or the complement thereof. Preferably, the computer system comprises an identifier which identifies features in said sequence. Further provided is a database comprising at least one nucleotide sequence in computer readable form wherein said nucleotide sequence is substantially similar to any one of SEQ ID NO:46, SEQ ID NO:48, 25 SEQ ID NO:55, or the complement thereof. The database, for example, carries out functions comprising determining homology, aligning sequences, adjusting sequence alignments, assembling sequences having overlapping sequence, predicting gene sequence, predicting intron borders, identifying motifs, identifying domains, identifying untranslated regulatory sequences, identifying 30 putative sequencing errors, carries out functional genomics analyses, or carries out shuffling of nucleotide sequences.

The invention also provides a method for generating nucleotide sequences encoding polypeptides having at least one region of homology to SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55, or the complement thereof. The method comprises shuffling an unmodified nucleotide sequence which is

5 identical or substantially identical to SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55, or the complement thereof. The resulting shuffled nucleotide sequence is expressed and a gene product encoded thereby is selected for altered activity as compared to the activity in a polypeptide encoded by SEQ ID NO:46, SEQ ID NO:48, or SEQ ID NO:55. A DNA molecule comprising a shuffled nucleotide

10 sequence obtainable or produced by the method is also provided. In one embodiment, the shuffled DNA molecule encodes a polypeptide having enhanced tolerance to an inhibitor of the polypeptide encoded by SEQ ID NO:46, SEQ ID NO:48, or SEQ ID NO:55. The shuffled DNA molecule may be operably linked to a promoter to form a chimeric molecule which is introduced

15 to a host cell, e.g., a plant cell.

Brief Description of the Figures

Figure 1 provides the structure of amino-acid activating modules identified in peptide synthetase genes (adapted from Stachelhaus and Marahiel, J. Biol. Chem., 270, 6163, 1995; Stachelhaus and Marahiel, FEMS Microbiol. Lett., 125, 3, 1995; Pospiech 1995, *supra*; Marahiel, 1997, *supra*). Figure 1A shows the domain arrangements in two types of modules. Structural variations in the first module (safB1) of the gene *safB* are also indicated below type I. Figure 1B shows the correlation between module types and the nature of residues

20 in two fungal peptides. Open box: type I module; filled box: type II module. Each peptide sequence is given below.

Figure 2 is a restriction map of the cloned sequences surrounding the tagged site. A 11.3 kb genomic region (thick line) was cloned and completely sequenced. The original REMI insertion point in the mutant R.C4.2696 is

30 indicated by a vertical arrow. The asterisks indicate two targeted integration sites in the wild type genome. Two open reading frames (in opposite directions),

ORF1 (*CPS1*, 5.4 kb) and ORF2 (*TES1*, 1.1 kb) are indicated by open boxes below the map (the positions of putative introns are indicated by vertical bars). Locations of seven overlapping plasmid clones used for sequencing are indicated by thin lines on the top of the map (filled triangles represent the vector sequence in each clone). Sequencing strategy is indicated by arrow above each clone line.

Figures 3A-C are schematic representations which show the characterization of modular structure of *CPS1*. Peptide synthetase and thioesterase are indicated by open boxes; shaded boxes inside indicate functional domains and modules; vertical bars in the shaded boxes indicate highly conserved core sequences. Figure 3A illustrates the general structure of bacterial and fungal peptide synthetases (adapted from Marahiel, 1997, *supra*). A peptide synthetase gene cluster is shown on the top. There can be one or more amino acid activating module (cyclosporine synthetase has 11) in each protein; some peptide synthetases have thioesterase domains (TE), which can be either integrated into modules or encoded by a separate gene. Each synthetase can have type I, type II or both modules. A type I (minimal) module is enlarged to show organization of core sequences and domains. Some peptide synthetases also have condensation or epimerization domains. Figure 3B illustrates the organization of saframycin Mx1 synthetase containing 4 amino acid activating modules (Pospiech et al., 1996, *supra*). SafB1 from the first module is enlarged. Core sequences 1 and 5 in safB1 are weakly conserved (indicated by dashed vertical bars). The remaining domains are typical of type I as shown in Figure 3A. SafC is a putative O-methyltransferase. Figure 3C illustrates the organization of *CPS1*. Sequence analysis revealed two amino acid activating modules (*CPS1A* and *CPS1B*), both of which have high similarity to safB1 except that core 2 is weakly conserved. A thioesterase domain is found at the C-terminal region of *CPS1B*. Three vertical arrows indicate the positions of targeted gene disruptions in the wild type genome that yielded the mutant phenotype. *TES1* is a thioesterase encoded by a separate gene (*TES1*).

Figures 4A-C depict DNA gel blots showing DNA-DNA hybridization of ChCPS1 to other fungal genera and species. (A) *Cochliobolus* species (1-17): *C.*

heterostrophus race T, race O; *C. carbonum* race 1, race 2; *C. victoriae* isolates FI3, HvW; *C. bicolor*, *C. dactyloctenii*, *C. chloridis*, *C. homomorphus*, *C. intermedius*, *C. melinidis*, *C. melinidis*, *C. peregrinensis*, *C. perotidis*, *C. ravenelii* and *C. sativus*. (B) Other Ascomycete genera (1-14): *C. carbonum* race1 (control), *Setosphaeria rostrata*, *Stemphylium* spp., *Pyrenophora tritici* *repentis*, *Bipolaris sacchari*, *Alternaria* spp., *A. solani*, *Nectria haematococca*, *Fusarium oxysporum*, *Glomerella* spp. *Magnaporthe grisea*, *F. moniliforme*, *F. moniliforme* (repeat) and *A. solani* (repeat). (C) *Candida albicans* compared to *C. heterostrophus* and closely related species (1-7): *C. heterostrophus* race T, *Bipolaris sacchari*, *Setosphaeria rostrata*, *Stemphylium* spp., *Pyrenophora tritici* *repentis*, *Alternaria* spp. and *Candida albicans* (arrowhead). Genomic DNAs were digested with *Hind*III (A, lanes 1-17; B, lanes 1-11; C, lanes 1-7), *Xho*I (B, lanes 12 and 14) or *Bgl*II (B, lane 13) and probed with the 3.2 kb fragment of CPS1 at high stringency. Weak signals in lanes 3 and 17 (panel A) are due to insufficient DNA loading (confirmed by a repeat experiment).

Figures 5A-B show similarity of the cloned CPS1 homologs to *C. heterostrophus* CPS1. (A) Structural comparison of the four CPS1 homologs to ChCPS1 (As = *Alternaria solani*; Pt = *Pyrenophora teres*; Fg = *Fusarium graminearum*; Ci = *Coccidioides immitis*). ORFs are indicated by the open boxes; shaded boxes inside indicate functional domains; vertical bars indicate conserved motif sequences found in nonribosomal peptide synthetases (NRPS) as defined by Stachelhaus and Marahiel (Stachelhaus and Marahiel, 1995, *supra*; Marahiel, 1997, *supra*) (dashed bars indicate weak conservation). The black bulbs indicate the position of putative introns. Cores 1-5: adenylation; core 6: thiolation; TE: thioesterase. The distance between core sequences is not drawn in exact scale. The name of proteins is on the left of ORF box and the number of amino acids on the right. The unidentified regions of AsCPS1, PtCPS1 and CiCPS1 are indicated by dash-lined boxes. The similarity to ChCPS1 (in the overlapping region only) is given in the parentheses under the protein names in the order: nucleotide identity/ amino acid identity/ amino acid similarity. The positions of the ChCPS1 amino acids 220 and 1040 (corresponding to the first

and the last amino acid of CiCPS1) are indicated by open arrows; the positions 511 and 1269 (to the first and the last amino acids of AsCPS1 and PtCPS1) are indicated by filled triangles. (B) Amino acid alignment of the four CPS1 homologs to ChCPS1. 530 amino acids aligned to the amino acids 511-1040 of ChCPS1 (SEQ ID NO:186) are shown (SEQ ID NOs: 51-54). The identical residues are in uppercase and the similar residues in lowercase. Consensus of sequences similar to the typical NRPS signature motifs is underlined. The putative cyclization domain motif “DXXXXD/ EXXS/ A” (SEQ ID NO:60) is underlined.

Figure 6 shows the results of a BLAST search using FgCPS1 (SEQ ID NO:41) as the query sequence.

Figure 7A shows the results of a BLAST search using CiCPS1 (SEQ ID NO:47) as the query sequence.

Figure 7B shows an alignment of amino acid sequence of FgCPS1 (SEQ ID NO:41), AsCPS1 (SEQ ID NO:43), PtCPS1 (SEQ ID NO:45), CiCPS1 (SEQ ID NO:47), and ChCPS1 (SEQ ID NO:3).

Figures 8A-C show the sequencing strategy (A), restriction map (B), genome organization (C) for the *ChCPS1* gene cluster. SEQ ID NO:59 represents the sequence of genes clustered near *ChCPS1*. SEQ ID NO:187 and 188 represent the DNA corresponding to and amino acid sequence encoded by ORF 16, respectively. SEQ ID NO:189 and 190 represent the DNA corresponding to and amino acid sequence corresponding to ORF 10, respectively. SEQ ID NO:191 and 192 represent the DNA corresponding to and amino acid sequence encoded by ORF 11, respectively. SEQ ID NO:193 and 194 represent the DNA corresponding to and amino acid sequence encoded by ORF 12, respectively. SEQ ID NO:195 and 196 represent the DNA corresponding to and amino acid sequence encoded by ORF 13, respectively. SEQ ID NO:197 and 198 represent the DNA corresponding to and amino acid sequence encoded by ORF 14, respectively. SEQ ID NO:199 and 200 represent the DNA corresponding to and amino acid sequence encoded by ORF 3, respectively. SEQ ID NO:201 and 202 represent the DNA corresponding to and

amino acid sequence encoded by ORF 5, respectively. SEQ ID NO:203 and 204 represent the DNA corresponding to and amino acid sequence encoded by ORF 6, respectively. SEQ ID NO:205 and 206 represent the DNA corresponding to and amino acid sequence encoded by ORF 7, respectively. SEQ ID NO:207 and 208 represent the DNA corresponding to and amino acid sequence encoded by ORF 8, respectively. SEQ ID NO:209 and 210 represent the DNA corresponding to and amino acid sequence encoded by ORF 9, respectively.

Figure 9A shows the results of a BLAST search using SEQ ID NO:49 (an iron reductase encoded by SEQ ID NO:48) as the query sequence.

Figure 9B shows an alignment of amino acid sequence of a *Cochliobolus* iron reductase (SEQ ID NO:49) and a *S. cerevisiae* reductase (SEQ ID NO:184).

Figure 9C illustrates a DNA comprising SEQ ID NO:48 (SEQ ID NO:211).

Figure 9D illustrates the amino acid sequence (SEQ ID NO:212) encoded by SEQ ID NO:211.

Figure 10 shows the results of a BLAST search using the polypeptide (SEQ ID NO:56) encoded by SEQ ID NO:55 (a *Cochliobolus* permease and/or MFS transporter) as the query sequence.

Detailed Description of the Invention

Definitions

The term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, composed of monomers (nucleotides) containing a sugar, phosphate and a base which is either a purine or pyrimidine. Unless specifically limited, the term encompasses nucleic acids containing known analogs of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated.

Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., Nucl. Acids Res., 19:508 (1991); Ohtsuka et al., JBC, 260:2605 (1985); Rossolini et al., Mol. Cell. Probes, 8:91 (1994). Although nucleotides are usually joined by phosphodiester linkages, polymeric nucleotides joined by peptide linkages (peptide nucleic acids) are also included (Nielsen and Egholm, Peptide Nucleotide Acids: Protocols and Applications, Horizon Scientific Press, Wymondham, Norfolk UK, 1999). A “nucleic acid fragment” is a fraction of a given nucleic acid molecule. Deoxyribonucleic acid (DNA) in the majority of organisms is the genetic material while ribonucleic acid (RNA) is involved in the transfer of information contained within DNA into proteins. The term “nucleotide sequence” refers to a polymer of DNA or RNA which can be single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases capable of incorporation into DNA or RNA polymers. The terms “nucleic acid”, “nucleic acid molecule”, “nucleic acid fragment” or “nucleic acid sequence or segment” may also be used interchangeably with gene, cDNA, DNA and RNA encoded by a gene.

The invention encompasses isolated or substantially purified nucleic acid or protein compositions. In the context of the present invention, an “isolated” or “purified” DNA molecule or an “isolated” or “purified” polypeptide is a DNA molecule or polypeptide that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated DNA molecule or polypeptide may exist in a purified form or may exist in a non-native environment such as, for example, a transgenic host cell. For example, an “isolated” or “purified” nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In one embodiment, an “isolated” nucleic acid is free of sequences that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the

genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. A protein that is substantially free of cellular material includes preparations of protein or polypeptide having less than about 30%, 20%, 10%, 5%, (by dry weight) of contaminating protein. When the protein of the invention, or biologically active portion thereof, is recombinantly produced, preferably culture medium represents less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein-of-interest chemicals. Fragments and variants of the disclosed nucleotide sequences and proteins or partial-length proteins encoded thereby are also encompassed by the present invention.

By "fragment" or "portion" is meant a full length or less than full length of the nucleic acid sequence encoding, or the amino acid sequence of, a polypeptide or protein. Alternatively, fragments or portions of a nucleotide sequence that are useful as hybridization probes generally do not encode fragment proteins retaining biological activity. Thus, fragments or portions of a nucleotide sequence may range from at least about 6 nucleotides, about 9, about 12 nucleotides, about 20 nucleotides, about 50 nucleotides, about 100 nucleotides or more. By "portion" or "fragment", as it relates to a nucleic acid molecule, sequence or segment of the invention, when it is linked to other sequences for expression, is meant a sequence having at least 80 nucleotides, more preferably at least 150 nucleotides, and still more preferably at least 400 nucleotides. If not employed for expressing, a "portion" or "fragment" means at least 6, about 9, preferably 12, more preferably 15, even more preferably at least 20, consecutive nucleotides, e.g., probes and primers (oligonucleotides), corresponding to the nucleotide sequence of the nucleic acid molecules of the invention.

By "resistant" is meant an organism, e.g., a plant or animal, that exhibits substantially no phenotypic changes as a consequence of infection with a

pathogen. By “tolerant” is meant an organism which, although it may exhibit some phenotypic changes as a consequence of infection, does not have a decreased reproductive capacity or substantially altered metabolism.

5 The term “gene” is used broadly to refer to any segment of nucleic acid associated with a biological function. Thus, genes include coding sequences and/or the regulatory sequences required for their expression. For example, gene refers to a nucleic acid fragment that expresses mRNA, functional RNA, or specific protein, including regulatory sequences. Genes also include nonexpressed DNA segments that, for example, form recognition sequences for
10 other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

“Naturally occurring” is used to describe an object that can be found in
15 nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

A “marker gene” encodes a selectable or screenable trait.

20 “Selectable marker” is a gene whose expression in a cell gives the cell a selective advantage. The selective advantage possessed by the cells transformed with the selectable marker gene may be due to their ability to grow in the presence of a negative selective agent, such as an antibiotic or a herbicide, compared to the growth of non-transformed cells. The selective advantage
25 possessed by the transformed cells, compared to non-transformed cells, may also be due to their enhanced or novel capacity to utilize an added compound as a nutrient, growth factor or energy source. Selectable marker gene also refers to a gene or a combination of genes whose expression in a cell gives the cell both a negative and/or a positive selective advantage.

30 The term “chimeric” refers to any gene or DNA that contains 1) DNA sequences, including regulatory and coding sequences, that are not found

together in nature, or 2) sequences encoding parts of proteins not naturally
adjoined, or 3) parts of promoters that are not naturally adjoined. Accordingly, a
chimeric gene may comprise regulatory sequences and coding sequences that are
derived from different sources, or comprise regulatory sequences and coding
5 sequences derived from the same source, but arranged in a manner different from
that found in nature.

A “transgene” refers to a gene that has been introduced into the genome
by transformation and is stably maintained. Transgenes may include, for
example, DNA that is either heterologous or homologous to the DNA of a
10 particular plant to be transformed. Additionally, transgenes may comprise native
genes inserted into a non-native organism, or chimeric genes. The term
“endogenous gene” refers to a native gene in its natural location in the genome of
an organism. A “foreign” gene refers to a gene not normally found in the host
organism but that is introduced by gene transfer.

15 The terms “protein,” “peptide” and “polypeptide” are used
interchangeably herein.

By “variants” is intended substantially similar sequences. For nucleotide
sequences, variants include those sequences that, because of the degeneracy of
the genetic code, encode the identical amino acid sequence of the native protein.
20 Naturally occurring allelic variants such as these can be identified with the use
of well-known molecular biology techniques, as, for example, with polymerase
chain reaction (PCR) and hybridization techniques. Variant nucleotide
sequences also include synthetically derived nucleotide sequences, such as those
generated, for example, by using site-directed mutagenesis which encode the
25 native protein, as well as those that encode a polypeptide having amino acid
substitutions. Generally, nucleotide sequence variants of the invention will have
at least 40, 50, 60, to 70%, e.g., preferably 71%, 72%, 73%, 74%, 75%, 76%,
77%, 78%, to 79%, generally at least 80%, e.g., 81%-84%, at least 85%, e.g.,
86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, to 98%,
30 sequence identity to the native (endogenous) nucleotide sequence.

“DNA shuffling” is a method to introduce mutations or rearrangements, preferably randomly, in a DNA molecule or to generate exchanges of DNA sequences between two or more DNA molecules, preferably randomly. The DNA molecule resulting from DNA shuffling is a shuffled DNA molecule that is a non-naturally occurring DNA molecule derived from at least one template DNA molecule. The shuffled DNA preferably encodes a variant polypeptide modified with respect to the polypeptide encoded by the template DNA, and may have an altered biological activity with respect to the polypeptide encoded by the template DNA.

The nucleic acid molecules of the invention can be optimized for enhanced expression in an organism of interest (Wada et al., Nucl Acids Res., 18:2367 (1990). For plants see, for example, EPA035472; WO91/16432; Perlak et al., Proc. Natl. Acad. Sci. USA, 88:3324 (1991); and Murray et al., Nucl Acids Res. 17:477 (1989). In this manner, the genes or gene fragments can be synthesized utilizing plant-preferred codons. See, for example, Campbell and Gowri, 1990 for a discussion of host-preferred codon usage. Thus, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic or partially optimized sequences may also be used.

Variant nucleotide sequences and proteins also encompass sequences and protein derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different coding sequences can be manipulated to create a new polypeptide possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer, Nature, 370:389 (1994); Cramer et al., Nature Biotech., 15:436 (1997); Moore et al., JMB, 272:336 (1997); Zhang et al., Proc. Natl. Acad. Sci. USA, 94:4504 (1997); Cramer et al., Nature, 391:288 (1998); and U.S. Patent Nos. 5,605,793 and 5,837,458.

“Conservatively modified variations” of a particular nucleic acid sequence refers to those nucleic acid sequences that encode identical or essentially identical amino acid sequences, or where the nucleic acid sequence does not encode an amino acid sequence, to essentially identical sequences.

5 Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance the codons CGT, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the
10 encoded protein. Such nucleic acid variations are “silent variations” which are one species of “conservatively modified variations.” Every nucleic acid sequence described herein which encodes a polypeptide also describes every possible silent variation, except where otherwise noted. One of skill will recognize that each codon in a nucleic acid (except ATG, which is ordinarily the
15 only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each “silent variation” of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

“Recombinant DNA molecule” is a combination of DNA sequences that are joined together using recombinant DNA technology and procedures used to
20 join together DNA sequences as described, for example, in Sambrook et al., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1989).

The terms “heterologous DNA sequence,” “exogenous DNA segment” or “heterologous nucleic acid,” each refer to a sequence that originates from a source foreign to the particular host cell or, if from the same source, is modified
25 from its original form. Thus, a heterologous gene in a host cell includes a gene that is endogenous to the particular host cell but has been modified through, for example, the use of DNA shuffling. The terms also include non-naturally occurring multiple copies of a naturally occurring DNA sequence. Thus, the terms refer to a DNA segment that is foreign or heterologous to the cell, or
30 homologous to the cell but in a position within the host cell nucleic acid in which

the element is not ordinarily found. Exogenous DNA segments are expressed to yield exogenous polypeptides.

A “microarray” as used herein is a solid support and a plurality of different oligonucleotides attached to the support. Each of the different
5 oligonucleotides is attached to the surface of the solid support in a different defined region, has a different determinable sequence, and is at least six nucleotides in length. Preferably, at least one of the different oligonucleotides is derived from a region of a polynucleotide having a nucleotide sequence selected from SEQ ID NO:46, SEQ ID NO:48 and SEQ ID NO:55, or the complement
10 thereof.

A “homologous” DNA sequence is a DNA sequence that is naturally associated with a host cell into which it is introduced.

“Wild-type” refers to the normal gene, e.g., a gene found in the highest frequency in a particular population, or organism found in nature without any
15 known mutation.

“Genome” refers to the complete genetic material of an organism.

“Vector” is defined to include, inter alia, any plasmid, cosmid, phage or binary vector in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable, and which can transform
20 prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally (e.g., autonomous replicating plasmid with an origin of replication).

Specifically included are shuttle vectors by which is meant a DNA vehicle capable, naturally or by design, of replication in two different host
25 organisms, which may be selected from actinomycetes and related species, bacteria and eukaryotic (e.g., higher plant, mammalian, yeast or fungal cells).

“Cloning vectors” typically contain one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences can be inserted in a determinable fashion without loss of essential biological function of the
30 vector, as well as a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically

include genes that provide tetracycline resistance, hygromycin resistance or ampicillin resistance.

“Expression cassette” as used herein means a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to the nucleotide sequence of interest which is operably linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The coding region usually codes for a protein of interest but may also code for a functional RNA of interest, for example antisense RNA or a nontranslated RNA, in the sense or antisense direction. The expression cassette comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development.

Such expression cassettes will comprise the transcriptional initiation region of the invention linked to a nucleotide sequence of interest. Such an expression cassette is provided with a plurality of restriction sites for insertion of the gene of interest to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

A transcriptional cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region, a DNA sequence of interest, and a transcriptional and translational termination region functional in plants. The termination region may be native with the transcriptional initiation region, may be native with the DNA sequence of interest, or may be derived from another source. For expression in plants,

convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also, Guerineau et al., Mol. Gen. Genetics, 262:141 (1991); Proudfoot, Cell, 64:671 (1991); Sanfacon et al., Genes Dev., 5:141 (1991);
5 Mogen et al., Plant Cell, 2:1261 (1990); Munroe et al., Gene, 91:151 (1990); Ballas et al., Nucl. Acids Res., 17:7891 (1989); Joshi et al., Nucl. Acids Res., 15:9827 (1987).

An oligonucleotide corresponding to a nucleic acid molecule of the invention may be about 30 or fewer nucleotides in length (e.g., 9, 12, 15, 18, 20,
10 21 or 24, or any number between 9 and 30). Generally specific primers are upwards of 14 nucleotides in length. For optimum specificity and cost effectiveness, primers of 16-24 nucleotides in length may be preferred. Those skilled in the art are well versed in the design of primers for use processes such as PCR. If required, probing can be done with entire restriction fragments of the
15 gene disclosed herein which may be 100's or even 1000's of nucleotides in length.

“Coding sequence” refers to a DNA or RNA sequence that codes for a specific amino acid sequence and excludes the non-coding sequences 5' and 3' to the coding sequence. It may constitute an “uninterrupted coding sequence”,
20 i.e., lacking an intron, such as in a cDNA or it may include one or more introns bounded by appropriate splice junctions, e.g., as may be found in genomic DNA. An “intron” is a sequence of RNA which is contained in the primary transcript but which is removed through cleavage and re-ligation of the RNA within the cell to create the mature mRNA that can be translated into a protein.

25 The terms “open reading frame” and “ORF” refer to the amino acid sequence encoded between translation initiation and termination codons of a coding sequence. The terms “initiation codon” and “termination codon” refer to a unit of three adjacent nucleotides (“codon”) in a coding sequence that specifies initiation and chain termination, respectively, of protein synthesis (mRNA
30 translation).

A “functional RNA” refers to an antisense RNA, ribozyme, or other RNA that is not translated.

The term “RNA transcript” refers to the product resulting from RNA polymerase catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA” (mRNA) refers to the RNA that is without introns and that can be translated into protein by the cell. “cDNA” refers to a single- or a double-stranded DNA that is complementary to and derived from mRNA.

“Regulatory sequences” and “suitable regulatory sequences” each refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences include enhancers, promoters, translation leader sequences, introns, and polyadenylation signal sequences. They include natural and synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences. As is noted above, the term “suitable regulatory sequences” is not limited to promoters. However, some suitable regulatory sequences useful in the present invention will include, but are not limited to constitutive promoters, tissue-specific promoters, development-specific promoters, inducible promoters and viral promoters.

“5' non-coding sequence” refers to a nucleotide sequence located 5' (upstream) to the coding sequence. It is present in the fully processed mRNA upstream of the initiation codon and may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency (Turner et al., Mol. Biotech., 3:225 (1995)).

“3' non-coding sequence” refers to nucleotide sequences located 3' (downstream) to a coding sequence and include polyadenylation signal sequences and other sequences encoding regulatory signals capable of affecting

mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al., Plant Cell, 1, 671, 1989.

5 “Promoter” refers to a nucleotide sequence, usually upstream (5') to its coding sequence, which controls the expression of the coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. “Promoter” includes a minimal promoter that is a short DNA sequence comprised of a TATA- box and other sequences that serve to
10 specify the site of transcription initiation, to which regulatory elements are added for control of expression. “Promoter” also refers to a nucleotide sequence that includes a minimal promoter plus regulatory elements that is capable of controlling the expression of a coding sequence or functional RNA. This type of promoter sequence consists of proximal and more distal upstream elements, the
15 latter elements often referred to as enhancers. Accordingly, an “enhancer” is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue specificity of a promoter. It is capable of operating in both orientations (normal or flipped), and is capable of functioning even when moved either
20 upstream or downstream from the promoter. Both enhancers and other upstream promoter elements bind sequence-specific DNA-binding proteins that mediate their effects. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even be comprised of synthetic DNA segments. A promoter may also
25 contain DNA sequences that are involved in the binding of protein factors which control the effectiveness of transcription initiation in response to physiological or developmental conditions.

 The “initiation site” is the position surrounding the first nucleotide that is part of the transcribed sequence, which is also defined as position +1. With
30 respect to this site all other sequences of the gene and its controlling regions are numbered. Downstream sequences (i.e. further protein encoding sequences in the

3' direction) are denominated positive, while upstream sequences (mostly of the controlling regions in the 5' direction) are denominated negative.

Promoter elements, particularly a TATA element, that are inactive or that have greatly reduced promoter activity in the absence of upstream activation are referred to as “minimal or core promoters.” In the presence of a suitable transcription factor, the minimal promoter functions to permit transcription. A “minimal or core promoter” thus consists only of all basal elements needed for transcription initiation, e.g., a TATA box and/or an initiator.

“Constitutive expression” refers to expression using a constitutive or regulated promoter. “Conditional” and “regulated expression” refer to expression controlled by a regulated promoter.

“Operably-linked” refers to the association of nucleic acid sequences on single nucleic acid fragment so that the function of one is affected by the other. For example, a regulatory DNA sequence is said to be “operably linked to” or “associated with” a DNA sequence that codes for an RNA or a polypeptide if the two sequences are situated such that the regulatory DNA sequence affects expression of the coding DNA sequence (i.e., that the coding sequence or functional RNA is under the transcriptional control of the promoter). Coding sequences can be operably-linked to regulatory sequences in sense or antisense orientation.

“Expression” refers to the transcription and/or translation of an endogenous gene or a transgene in plants. For example, in the case of antisense constructs, expression may refer to the transcription of the antisense DNA only. In addition, expression refers to the transcription and stable accumulation of sense (mRNA) or functional RNA. Expression may also refer to the production of protein.

“Altered levels” refers to the level of expression in transgenic cells or organisms that differs from that of normal or untransformed cells or organisms.

“Overexpression” refers to the level of expression in transgenic cells or organisms that exceeds levels of expression in normal or untransformed cells or organisms.

“Antisense inhibition” refers to the production of antisense RNA transcripts capable of suppressing the expression of protein from an endogenous gene or a transgene.

5 “Co-suppression” and “transwitch” each refer to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar transgene or endogenous genes (U.S. Patent No. 5,231,020).

“Gene silencing” refers to homology-dependent suppression of viral genes, transgenes, or endogenous nuclear genes. Gene silencing may be transcriptional, when the suppression is due to decreased transcription of the
10 affected genes, or post-transcriptional, when the suppression is due to increased turnover (degradation) of RNA species homologous to the affected genes (English et al., Plant Cell, 8:179 (1996). Gene silencing includes virus-induced gene silencing (Ruiz et al., Plant Cell, 10:937 (1998).

“Chromosomally-integrated” refers to the integration of a foreign gene or
15 DNA construct into the host DNA by covalent bonds. Where genes are not “chromosomally integrated” they may be “transiently expressed.” Transient expression of a gene refers to the expression of a gene that is not integrated into the host chromosome but functions independently, either as part of an autonomously replicating plasmid or expression cassette, for example, or as part
20 of another biological system such as a virus.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) “reference sequence”, (b) “comparison window”, (c) “sequence identity”, (d) “percentage of sequence identity”, and (e) “substantial identity”.

25 (a) As used herein, “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, “comparison window” makes reference to a
30 contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or

deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art
5 understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can
10 be accomplished using a mathematical algorithm. Preferred, non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller, CABIOS, 4:11 (1988); the local homology algorithm of Smith et al., Adv. Appl. Math., 2:482 (1981); the homology alignment algorithm of Needleman and Wunsch, JMB, 48:443 (1970); the search-for-similarity-method
15 of Pearson and Lipman, Proc. Natl. Acad. Sci. USA, 85:2444 (1988); the algorithm of Karlin and Altschul, Proc. Natl. Acad. Sci. USA, 87:2264 (1990), modified as in Karlin and Altschul, Proc. Natl. Acad. Sci. USA, 90:5873 (1993).

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such
20 implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 8 (available from Genetics Computer Group (GCG), 575 Science Drive, Madison, Wisconsin, USA).
25 Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins et al., Gene, 73:237 (1988); Higgins et al., CABIOS, 5:151 (1989); Corpet et al., Nucl. Acids Res., 16:10881 (1988); Huang et al., CABIOS, 8:155 (1992); and Pearson et al., Meth. Mol. Biol., 24:307 (1994). The ALIGN program is based on the algorithm of
30 Myers and Miller, *supra*. The BLAST programs of Altschul et al., JMB,

215:403 (1990); Nucl. Acids Res., 25:3389 (1990), are based on the algorithm of Karlin and Altschul *supra*.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information

5 (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., 1990,

10 *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and

15 N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-

20 scoring residue alignments, or the end of either sequence is reached.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993), *supra*). One measure of similarity provided by the BLAST algorithm is the smallest sum probability

25 (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more

30 preferably less than about 0.01, and most preferably less than about 0.001.

To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul et al., 1997. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul et al.,
5 *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g. BLASTN for nucleotide sequences, BLASTX for proteins) can be used. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid
10 sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, 1989). See <http://www.ncbi.nlm.nih.gov>. Alignment may also be performed manually by inspection.

For purposes of the present invention, comparison of nucleotide
15 sequences for determination of percent sequence identity to the sequences disclosed herein is preferably made using the BlastN program (version 1.4.7 or later) with its default parameters or any equivalent program. By “equivalent program” is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or
20 amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by the preferred program.

(c) As used herein, “sequence identity” or “identity” in the context of two nucleic acid or polypeptide sequences makes reference to a specified percentage of residues in the two sequences that are the same when aligned for maximum
25 correspondence over a specified comparison window, as measured by sequence comparison algorithms or by visual inspection. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with
30 similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in

conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have “sequence similarity” or “similarity.” Means for making this adjustment are well known to those of skill
5 in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is
10 calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, “percentage of sequence identity” means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison
15 window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions,
20 dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

(e)(i) The term “substantial identity” of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70%, 71%, 72%,
25 73%, 74%, 75%, 76%, 77%, 78%, or 79%, preferably at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more preferably at least 90%, 91%, 92%, 93%, or 94%, and most preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill in the art will
30 recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by

taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 70%, more preferably at least 80%, 90%, and most preferably at least 95%.

5 Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions (see below). Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. However, stringent conditions encompass temperatures in the range of
10 about 1°C to about 20°C, depending upon the desired degree of stringency as otherwise qualified herein. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides they encode are substantially identical. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic
15 code. One indication that two nucleic acid sequences are substantially identical is when the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

(e)(ii) The term “substantial identity” in the context of a peptide indicates that a peptide comprises a sequence with at least 70%, 71%, 72%, 73%, 74%,
20 75%, 76%, 77%, 78%, or 79%, preferably 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more preferably at least 90%, 91%, 92%, 93%, or 94%, or even more preferably, 95%, 96%, 97%, 98% or 99%, sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of
25 Needleman and Wunsch, 1970, *supra*. An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution.

30 For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence

comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

As noted above, another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA. "Bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target nucleic acid sequence.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. Longer sequences hybridize specifically at higher temperatures. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, 1984; $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\%\text{-form}) - 500/L$; where M is the molarity of monovalent cations, $\%GC$ is the percentage of guanosine and cytosine nucleotides in the DNA, $\%\text{-form}$ is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with

>90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, 1993. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH.

Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or Northern blot is 50% formamide, e.g., hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C.

The following are examples of sets of hybridization/wash conditions that may be used to clone orthologous nucleotide sequences that are substantially

identical to reference nucleotide sequences of the present invention: a reference nucleotide sequence preferably hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

By "variant" polypeptide is intended a polypeptide derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Such variants may results form, for example, genetic polymorphism or from human manipulation. Methods for such manipulations are generally known in the art.

Thus, the polypeptides of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the polypeptides can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel, Proc. Natl. Acad. Sci. USA, 82:488 (1985); Kunkel et al., Meth. Enzymol., 154:367 (1987); U. S. Patent No. 4,873,192; Walker and Gaastra, Techniques in Mol. Biol. (MacMillan Publishing Co. (1983), and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff et al., Atlas of Protein Sequence and

Structure (Natl. Biomed. Res. Found. 1978). Conservative substitutions, such as exchanging one amino acid with another having similar properties, are preferred.

Thus, the genes and nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms. Likewise, the polypeptides of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. Such variants will continue to possess the desired activity. The deletions, insertions, and substitutions of the polypeptide sequence encompassed herein are not expected to produce radical changes in the characteristics of the polypeptide. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays.

Individual substitutions deletions or additions that alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are “conservatively modified variations,” where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following five groups each contain amino acids that are conservative substitutions for one another: Aliphatic: Glycine (G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I); Aromatic: Phenylalanine (F), Tyrosine (Y), Tryptophan (W); Sulfur-containing: Methionine (M), Cysteine (C); Basic: Arginine (R), Lysine (K), Histidine (H); Acidic: Aspartic acid (D), Glutamic acid (E), Asparagine (N), Glutamine (Q). See also, Creighton, 1984. In addition, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids in an encoded sequence are also “conservatively modified variations.”

“Germline cells” refer to cells that are destined to be gametes and whose genetic material is heritable.

The word “plant” refers to any plant, particularly to seed plant, and “plant cell” is a structural and physiological unit of the plant, which comprises a cell

wall but may also refer to a protoplast. The plant cell may be in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, or a plant organ.

5 “Plant tissue” includes differentiated and undifferentiated tissues or plants, including but not limited to roots, stems, shoots, leaves, pollen, seeds, tumor tissue and various forms of cells and culture such as single cells, protoplast, embryos, and callus tissue. The plant tissue may be in plants or in organ, tissue or cell culture.

10 The term “altered plant trait” means any phenotypic or genotypic change in a transgenic plant relative to the wild-type or non-transgenic plant host.

15 The term “transformation” refers to the transfer of a nucleic acid fragment into the genome of a host cell, resulting in genetically stable inheritance. Host cells containing the transformed nucleic acid fragments are referred to as “transgenic” cells, and organisms comprising transgenic cells are referred to as “transgenic organisms”. Examples of methods of transformation of plants and plant cells include *Agrobacterium*-mediated transformation (De Blaere et al., Meth. Enzymol., 143:277 (1987) and particle bombardment technology (Klein et al., Nature, 327:70 (1987); U.S. Patent No. 4,945,050). Whole plants may be regenerated from transgenic cells by methods well known to the skilled artisan (see, for example, Fromm et al., Biotech., 8:833 (1990)).

20 “Transformed,” “transgenic,” and “recombinant” refer to a host cell or organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome generally known in the art and are disclosed in 25 Sambrook et al., 1989, *supra*. See also Innis et al., PCR Protocols, Academic Press (1995); and Gelfand, PCR Strategies, Academic Press (1995); and Innis and Gelfand, PCR Methods Manual, Academic Press (1999). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, 30 vector-specific primers, partially mismatched primers, and the like. For example, “transformed,” “transformant,” and “transgenic” plants or calli have

been through the transformation process and contain a foreign gene integrated into their chromosome. The term “untransformed” refers to normal plants that have not been through the transformation process.

5 A “transgenic” organism is an organism having one or more cells that contain an expression vector.

“Transiently transformed” refers to cells in which transgenes and foreign DNA have been introduced but not selected for stable maintenance.

“Stably transformed” refers to cells that have been selected and regenerated on a selection media following transformation.

10 “Genetically stable” and “heritable” refer to chromosomally-integrated genetic elements that are stably maintained in the plant and stably inherited by progeny through successive generations.

“Enzyme activity” means herein the ability of an enzyme to catalyze the conversion of a substrate into a product. A substrate for the enzyme comprises
15 the natural substrate of the enzyme but also comprises analogues of the natural substrate which can also be converted by the enzyme into a product or into an analogue of a product. The activity of the enzyme is measured for example by determining the amount of product in the reaction after a certain period of time, or by determining the amount of product in the reaction after a certain period of
20 time, or by determining the amount of substrate remaining in the reaction mixture after a certain period of time. The activity of the enzyme is also measured by determining the amount of an unused co-factor of the reaction remaining in the reaction mixture after a certain period of time or by determining the amount of used co-factor in the reaction mixture after a certain period of
25 time. The activity of the enzyme is also measured by determining the amount of a donor of free energy or energy-rich molecule (e.g., ATP, phosphoenolpyruvate, acetyl phosphate or phosphocreatine) remaining in the reaction mixture after a certain period of time or by determining the amount of a used donor of a free energy or energy-rich molecule (e.g., ADP, pyruvate, acetate or creatine) in the
30 reaction mixture after a certain period of time.

“Fungicide” is a chemical substance used to kill or suppress the growth of fungal cells.

An “inhibitor” is a chemical substance that causes abnormal growth, e.g., by inactivating the enzymatic activity of a protein such as biosynthetic enzyme, receptor, signal transduction protein, structural gene product, or transport protein that is essential to the growth or survival, or alters the virulence or pathogenicity, of the fungus. In the context of the instant invention, an inhibitor is a chemical substance that alters the activity encoded by any one of SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:56 or their orthologs.

“Isogenic” fungi are genetically identical, except that they may differ by the presence or absence of a heterologous DNA sequence.

A “substrate” is the molecule that an enzyme naturally recognizes and converts to a product in the biochemical pathway in which the enzyme naturally carries out its function, or is a modified version of the molecule, which is also recognized by the enzyme and is converted by the enzyme to a product in an enzymatic reaction similar to the naturally-occurring reaction.

“Tolerance” as used herein is the ability of an organism, e.g., a fungus, to continue essentially normal growth or function when exposed to an inhibitor or fungicide in an amount sufficient to suppress the normal growth or function of native, unmodified fungi.

The Nucleic Acid Molecules of the Invention and Uses Thereof

The involvement of peptide synthetase genes in fungal pathogenesis to plants has been genetically tested only in two previous studies. In *C. carbonum*, disruption of both copies of the *HTS1* gene, which encodes HC-toxin synthetase, caused loss of ability to make HC-toxin and the fungus became nonpathogenic on HC-toxin sensitive corn plants (Panaccione et al, PNAS, 89, 6590, 1992), indicating that the HC-toxin synthetase gene is a pathogenicity determinant. In *Fusarium avenaceum*, the enniatin-nonproducing transformants were obtained by disruption of enniatin synthetase encoding gene (*esyn1*) and these transformants displayed significantly reduced virulence in a potato tuber tissue assay

(Herrmann et al., 1996) indicating that enniatin synthetase gene is a virulence factor in pathogenesis by the fungus. In these two pathosystems, only one fungal secondary metabolite (the peptide toxin) was studied. In contrast, the polyketide T-toxin has been well studied in *C. heterostrophs* and has been confirmed to be a host-specific virulence factor (Yoder and Turgeon, 1996; Yoder et al., 1997, *supra*) and this study demonstrated that a second secondary metabolite, the hypothetical CPS1 toxin is also involved in pathogenesis by the fungus. Unlike the T-toxin biosynthetic genes such as *PKS1* and *DECI* that are found only in race T (Yang et al., 1996, *supra*; Rose et al., 1996, *supra*), *CPS1* is found in both race O and race T. Disruption of *CPS1* in either race causes dramatically reduced fungal virulence as tested on N-cytoplasm corn. This result suggests that CPS1 toxin could be the same as the “race O” toxin proposed previously (Yoder, 1981). However, as disclosed herein, CPS1 is a CoA ligase.

Interestingly, a *Tox*⁺, *cps1*⁻ mutant also show reduced virulence on T-cytoplasm corn although it produced the same amount of T-toxin as wild type race T. This is unusual because the interaction between T-toxin and the T-corn-unique URF13 protein is highly specific; the same outcomes should be expected if two strains that produce the same amount of T-toxin attack the same host, T-corn. The most likely explanation for this result is that the fungal growth *in planta* has been inhibited by the host plant and the poor growth results in reduced T-toxin production which is normal when the fungus is grown in culture. Reduced virulence on T-cytoplasm corn is due to the reduced T-toxin production as that seen in leaky *Tox*⁻ mutants. This inhibition of growth could be due to the failure of suppression of the host defense mechanism by the fungus, which is mediated by the *CPS1* controlled peptide toxin. A *cps1*⁻ mutant that fails to produce this “suppressor” could not be able to colonize plant tissues as vigorously as wild type does, resulting in the reduced ability to cause disease as indicated by the smaller lesion phenotype. If this turns out to be the case, *CPS1* should be considered as a general virulence factor as proposed for enniatin.

It is possible that *cps1*⁻ mutants are still be able to produce a certain amount of CPS1 toxin. One probability is the gene has not been completely

inactivated by insertional mutagenesis or targeted disruption. The original REMI insertion occurred at core sequence 1 of CPS1A, a region that might be not critical (function of core 1 is unknown). The second targeted site is located between cores 1 and 2 of CPS1B and the third is located between cores 2 and 3 of the same module. All three insertions do not disrupt critical motifs. On the other hand, *CPSI* contains a number of in-frame start codons and some of them are located immediately downstream of these insertion sites. It is possible that each of these disruptions actually resulted in two subtranscripts, one is transcribed normally from the start codon of *CPSI* and stops at the insertion site and second is transcribed near one of these in-frame ATGs downstream of the insertion site and stops at the end of *CPSI*. Both transcripts could give a truncated protein that still has enzymatic activities. But these separate enzymes might have affinities for their substrates lower than that of holoenzyme. The reduced production of CPS1 toxin might be due to the CPS1 holoenzyme having been split into two fractions by the vector insertion and the resulting truncated proteins being much less active than the original polypeptide. This hypothesis can be tested by construction a *C. heterostrophus* strain in which the entire *CPSI* encoding sequence has been deleted.

The second possibility is the existence of multiple copies of *CPSI* in the genome. Previous studies have demonstrated that the gene encoding HC-toxin synthetase (*HTSI*) is duplicated in the genome and both copies (*HTSI-1* and *HTSI-2*) are 270 kb apart in most Tox2⁺ isolates of *C. carbonum* (Ahn and Walton, Plant Cell, 8, 887, 1996). Disruption of either copy reduced HTS1 activity but did not affect HC-toxin production; when both copies were

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disrupted, HC-toxin production was abolished (Panaccione et al, 1992, *supra*). But in contrast to the case of *HTSI*, gel blot analysis does not indicate the presence of a second copy of *CPS1* and disruption of *CPS1* does affect the production of the putative toxin. It is unlikely that two genes with similar organization are in the genome. An alternative postulation is that there may be a second gene which encodes a protein with the same enzyme activity as *CPS1* but does not have significant sequence homology to *CPS1*. This hypothesis is hard to test unless this gene is clustered with *CPS1* and can be recovered by chromosome walking.

10 Pathogenesis by *C. heterostrophus* to corn involves at least two secondary metabolites: the T-toxin, a host specific factor which determines high virulence on a particular host, T-corn and the hypothetical *CPS1* toxin, a general factor (either virulence or pathogenicity factor) which contributes to basic mechanisms underlying the disease establishment by the fungus in common host plants.

15 By genomic DNA hybridization, *C. heterostrophus CPS1* homologs were found in 16 additional fungal species belonging to 5 genera. Hybridization signals for some were as strong as the *C. heterostrophus* gene, indicating that *CPS1* is highly conserved among these fungi. This conservation appears to match the taxonomic relationships between these species. *Cochliobolus* (anamorph *Bipolaris*) and *Setosphaeria* (anamorph *Exserohilum*) are closely related genera.

20 Two species, *C. victoriae* and *C. carbonum*, which are able to cross to each other and thus may not be different species (Scheffer et al., 1967; Yoder et al., 1989), showed the same hybridization pattern to *CPS1*. *B. sacchari*, the closest asexual relative of *C. heterostrophus*, hybridized to two *HindIII* fragments that were only seen in *C. heterostrophus* itself, but all other species gave only one distinct polymorphic band. Phylogenetic analyses using the internal transcribed spacer (ITS) sequences and fragments of the *GPD* (vanWert and Yoder, 1992) and *MAT* genes (Turgeon et al., Mol. Gen. Genet., 238, 270, 30 1993) also put *C. victoriae/C. carbonum* and *C. heterostrophus/B. sacchari*

closest to each other (Turgeon and Berbee, 1997). These results might imply that *CPSI* has coevolved with these genes.

The genera *Cochliobolus* and *Setosphaeria* include many plant pathogenic species that are commonly associated with leaf spots or blights, mainly on cultivated cereals and wild grasses (Sivanesan, 1987; Alcorn, 1988). This group of phytopathogenic fungi includes both mild pathogens and severe pathogens that often produce host-specific toxins (Yoder, 1980, *supra*). One of the essential questions is whether or not the various diseases on diverse host plants caused by these fungi involve common factors or depend only on individual specific factors, such as host-specific toxins.

Previous studies have shown that host-specific toxins can be critical factors for determining either virulence or host-range, but they do not account for general pathogenicity since they are produced only by certain isolates in the species and the corresponding biosynthetic genes are found only in these toxin-producing isolates (Yoder et al., 1997, *supra*). In contrast, *CPSI* homologs are found in all *Cochliobolus* and *Setosphaeria* species tested so far, suggesting they are a common factor shared by this group. Disruption of the *CPSI* homolog in the oat pathogen *C. victoriae* caused dramatically reduced virulence to victorin-susceptible oats although the transformants produced wild type levels of victorin. This result is similar to that with *C. heterostrophus* race T, in which *cpsI*⁻ disruptants still produced wild type levels of T-toxin but showed reduced virulence on T-cytoplasm corn. These results argue strongly that host-specific toxins alone are not sufficient in determining the ultimate outcome of fungus/plant interactions and suggest that the establishment of disease by these fungi also requires CPS1, which might control a pathway for general pathogenicity.

In the early 1990s, studies on pathogenesis by uropathogenic *E. coli* led to the identification of pathogenicity gene clusters, termed “pathogenicity islands” (Hecker et al., 1990; Blum et al., 1994). Subsequently, similar gene clusters were identified in additional animal or human bacterial pathogens, including *Yersinia pestis*, *Helicobacter pylori* and *Salmonella typhimurium*.

These islands often contain genes for production of toxins or genes encoding proteins that are capable of interacting with host defense factors or required for type III secretion systems that deliver virulence proteins into host cells. Usually, they are found only in pathogenic strains (or species); in rare cases, they occur in nonpathogenic strains of the same species or related species (Hacker et al., Mol. Microbiol., 23, 1089, 1997).

In phytopathogenic bacteria, *hrp* gene clusters have been referred to as “pathogenicity islands” because they have several features in common with “pathogenicity islands” in animal pathogenic bacteria, i.e., they are found only in pathogenic species (required for plant pathogenicity) and contain highly conserved genes (*hrc* genes) defining the type III protein secretion system (Alfano and Collmer, 1996; Barinaga, 1996).

In plant pathogenic fungi, genes or gene clusters with characteristics of “pathogenicity islands” have been identified from certain species, i.e., in *Nectria haematococca*, the *PDA* genes for detoxifying the pea phytoalexin and other pea pathogenicity genes (*PEP*) are located on dispensable chromosomes that are found in all isolates pathogenic to pea but usually absent in all nonpathogenic isolates (VanEtten et al., Antonie Van Leeuwenhoek, 65, 263, 1994; Liu et al., 1997, *supra*). In the genus *Cochliobolus*, the *Tox2* gene cluster controlling the biosynthesis of HC-toxin is found only in *C. carbonum* race 1 (pathogenic to *hm1hm1* corn) and the *Tox1* genes controlling T-toxin production are found only in *C. heterostrophus* race T (highly virulent on T-cytoplasm corn); all other races of the same species and all other fungal species tested so far lack these *Tox* genes (Ahn and Walton, 1996, *supra*; Yang et al., 1996, *supra*; Yoder et al., 1997, *supra*).

CPS1 differs in two important ways compared to these fungal “pathogenicity islands”. First, it is highly conserved among several phytopathogenic *Cochliobolus* species and relatives. Second, like certain bacterial “pathogenicity islands”, *CPS1* also has homologs in “nonpathogenic” species. *C. homomorphus* and *C. dactyloctenii*, neither of which causes disease on plants, hybridized strongly to *CPS1*. This may reflect genetic changes in the

“pathogenicity island” that resulted in loss of pathogenicity. In the bacterial genus *Listeria*, which includes several human or animal pathogenic species harboring highly conserved “pathogenicity islands”, the “pathogenicity island” homolog in the nonpathogenic species (*L. seeligeri*) was found to be “silent” due to a mutation that occurred in the promoter region of a critical regulatory gene in the cluster (Hacker et al., 1997, *supra*). These features suggest that the *CPSI* gene cluster and homologs could define a new group of fungal “pathogenicity islands”.

It is known that the evolution of pathogenicity involves two major processes. A pathogenic microorganism could originate from nonpathogenic progenitors by slow modifications (such as point mutations and genetic recombination) of genes that were adapted for parasitic growth on hosts or by the integration of large fragments of “alien” DNA into the genome that enable the recipient to attack particular hosts (gene horizontal transfer). The latter can occur in the recent or distant evolutionary past. Subsequent vertical transmission in the lineage (if the transferred gene is stable in the recipient genome) would result in the preserve of the gene in all species that diverged after the acquisition of the gene(s) (Scheffer, 1991; Arber, *Gene*, 135, 49, 1993; Krishnapillai, 1996; Burdon and Silk, 1997).

In the past few years, substantial evidence has become available that supports the hypothesis of gene horizontal transfer. All “pathogenicity islands” in animal pathogenic bacteria are believed to have been acquired by a horizontal transfer event (recent or past) because they usually differ in G+C content from the recipient genome and have transposable elements at the boundaries of the gene clusters (Hacker et al., 1997, *supra*). The *hrp* “pathogenicity islands” do not show a significant difference in G+C content or association with transposable elements, but they are also believed to have arisen similarly because *hrc* genes in these “pathogenicity islands” show high similarity to genes defining the type III protein secretion system found in animal pathogenic bacteria as mentioned above (Alfano and Collmer, 1996; and Barinaga, 1996).

Although *CPSI* itself has several typical fungal introns and a G+C content (51.5%) similar to most known fungal genes, genomic regions (about 1.5 kb) flanking the gene have higher G+C content (>60%). Several short G+C-rich regions are also found in the gene cluster; one of the open reading frames (ORF10) has a 63.6 % G+C content. Compared to those filamentous fungal genomes characterized so far, including *N. crassa*, *A. nidulans*, *U. maydis* (all have G+C content 51-54%, see Karlin and Mrázek, PNAS, 94, 10227, 1997), the genomic region around *CPSI* is unusual. This might suggest that the gene cluster harboring *CPSI* came from a bacterial source (since most bacterial genes are known to have a high G+C content), but has evolved into a fungal version.

Based on these data, *CPSI* homologs may have a common ancestral gene which was acquired from a bacterial species *via* horizontal transfer and then maintained by the fungal genome *via* vertical transmission in closely related lineages.

In the evolution process, the genus *Cochliobolus* could also have inherited a second gene (*X*) controlling the ability to take up foreign DNA, by which its ancestor took the “alien” *CPSI*. As a result, this group of fungi is able to keep trapping genes from other organisms by additional “horizontal transfers” and giving rise to new races or even new species characterized by the ability to produce unique pathogenesis factors. The direct support for this hypothesis is that both the *Tox2* locus of *C. carbonum* and the *Tox1* locus of *C. heterostrophus* are associated with large fragments of “alien” DNA (A+T-rich and highly repeated) and the same could also be true for *Tox3* controlling victorin production by *C. victoriae*, although there is yet no direct experimental evidence (Ahn and Walton, 1996, *supra*; Yang et al., 1996, *supra*; Yoder et al., 1997, *supra*). In contrast to *CPSI*, these gene transfers must have occurred in the recent evolutionary past because both *Tox1* and *Tox2* loci are found only in specific isolates in the species, e.g., the acquisition of *Tox1* genes probably occurred as recently as the 1960s when race T was first identified in the field (Yoder et al., 1997, *supra*).

There are other possibilities for the evolution of *CPSI*. First, each genus mentioned above could have acquired *CPSI* independently after divergence of the lineage. But this seems less likely because this would need to happen at the same time and involve the same donor organism if the fact that the homologs
5 detected in *Cochliobolus* and *Setosphaeria* gave similar hybridization signal intensity is considered. Second, the horizontal transfer of *CPSI* could have occurred at earlier time periods such as before the divergence of Pleosporales or even the Ascomycotina. To test these hypotheses, detection of *CPSI* homologs in *Pyrenophora*, *Pleospora* and other genera must be done by either genomic
10 DNA hybridization or PCR. Based on the facts discussed here, it is not unreasonable to predict that additional *CPSI* homologs will be found in other fungal species. Further investigation could provide an direct entry point for understanding the evolution of fungal pathogenesis to plants.

The *C. heterostrophus CPSI* gene was cloned by identification of
15 genomic DNA fragments recovered from the tagged site in a mutant generated using REMI insertional mutagenesis. Characterization of two overlapping cosmid clones in this study has proved that no deletions or chromosome rearrangements are associated with the gene tagging event, because both cosmids carry the same fragment which span the REMI insertion site and the nucleotide
20 sequence in this region is the same as that of recovered genomic DNA from the tagged site. This undoubtedly clarifies the identity of *CPSI*, which is the major biosynthetic gene. Mapping and sequencing of the two cosmids extended the sequence by 27.4 kb from the previously cloned fragment, leading to the characterization of 38.7 kb of contiguous genomic DNA, the largest genomic
25 region analyzed so far in *C. heterostrophus*. In addition to *CPSI* and *TES1*, sequence analysis of this region revealed at least 11 open reading frames; three of them, designated as *DBZ1*, *CAT1* and *DEC2*, respectively, apparently encode functional proteins. The tight linkage of these genes suggests that they may be involved in the same pathway.

30 In filamentous fungi, in some cases, genes in pathways for biosynthesis of secondary metabolites are dispersed on different chromosomes, e.g., the

cephalosporin C pathway genes in *Acremonium chrysogenum* (Mathison et al., Curr. Genet., 23, 33, 1993) and the melanin pathway genes in *Colletotrichum lagenarium* (Kubo et al., Appl. Environ. Microbiol., 62, 4340, 1996). In other cases, tightly linked genes are usually found to be functionally related to a common pathway. This clustering organization has been exemplified by the sterigmatocystin pathway genes of *Aspergillus nidulans*, in which coordinately regulated transcripts are found in a 60 kb genomic region (Brown et al., 1996) and the trichothecene pathway genes of *Fusarium sporotrichioides*, in which 9 genes are clustered in a 25 kb region and 8 of them have been shown to be required for the pathway function (Hohn et al., Mol. Gen. Genet., 248, 95, 1995). The genes involved in biosynthesis of certain fungal peptides are also found as clusters. The tight linkage between *CPSI* and these additional genes might reveal the presence of a novel secondary metabolite pathway in *C. heterostrophus*. In this pathway, *CPSI* is the major structural gene since it encodes a large multifunctional enzyme with all catalytic activities required for synthesis of a secondary metabolite, presumably a peptide phytotoxin; other genes may carry out different functions required for coordinate operation of the pathway, such as regulation, posttranslational modification or substrate processing as discussed below.

Both functional and structural analyses strongly support the hypothesis that the *CPSI* gene cluster controls a novel biosynthetic pathway. Pathway genes have been studied only in a few filamentous fungi mainly for industrial purposes (Keller et al., J. Ind. Microbiol. Biotechnol., 19, 305, 1997). For plant pathogenic fungi, little is known about pathway genes for fungal pathogenesis. In *C. heterostrophus*, recent cloning of two *ToxI* genes *PKSI* (Yang et al., 1996, *supra*) and *DEC1* (Rose et al., 1996, *supra*) have contributed to a breakthrough in understanding the molecular mechanism for biosynthesis of T-toxin, a virulence determinant in the fungus/corn interaction. But further identification of related pathway genes has been unsuccessful because the two genes are located on different chromosomes and each is embedded in A+T-rich DNA

(Yoder et al., 1997, *supra*). In contrast, the *CPSI* cluster provides a good opportunity to explore a pathogenesis pathway.

First, it resides in a “normal” sequence region. G+C content of a 50-55% is found in most of the cloned sequences and no A+T-rich DNA is associated with either end of the cloned region. This would facilitate cloning of additional pathway genes by further chromosome walking, by screening of cosmid libraries or the targeted integration and plasmid rescue. Second, it contains a regulatory gene (*DBZI*) which is presumably linked to a signal transduction pathway. Isolation of genes that interact with *DBZI* could reveal novel factors mediating the molecular communication between fungal pathogen and the host plant. Further characterization of *DBZI* (along with position-specific disruption or deletion) would be also helpful in determining the limit of the gene cluster, because tightly linked genes involved in a common pathway are often coordinately regulated by the same regulatory factor (Keller et al., 1997, *supra*). Finally, *CPSI* genes are found in both race T and race O, and its homologs are also found in other *Cochliobolus* species. Presence of high G+C content may imply that these genes evolved from a bacterial ancestor and the conservation in these fungi may correlate with the phytopathogenic function of the gene products encoded by the *CPSI* cluster. Further investigation of this cluster should provide insights into the evolution of general pathogenicity factors among this group of fungi.

Ferric reductases are a group of enzymes found in bacteria, fungi, plants and animals that are responsible for reduction of ferric iron to ferrous iron, an absorptive form used by the organism. They have been well studied in *S. cerevisiae*, *C. albicans* and *H. capsulatum* and the like. The yeast FER1 has been expressed in tobacco (Oki et al., 1999).

Previous studies have shown that FER genes could be important pathogenic determinants. Timmerman and Woods have proposed that in *H. capsulatum* FER could play critical roles in the acquisition of iron in three different ways: from inorganic or organic ferric salts, from host Fe(III) binding

proteins (transferrin and the like), and from siderophores produced by the fungus itself (to reduce and release the iron chelated by the siderophore molecules).

On the other hand, iron sequestration in response to microbial infection has been demonstrated to be a host defense mechanism. The infection-related iron acquisition system in the pathogen can be considered to be an important mechanism against host defense and for a successful colonization by the pathogen in the host cells. This could be a general mechanism for all pathogenic fungi.

CPS1 does encode a peptide synthetase which is responsible for biosynthesis of a novel siderophore with unusual amino acid, hydroxyl acid and architecture, which is why CPS1 does not show similarity to common NRPSs. The CPS1 siderophore can compete with the host for iron acquisition when the fungus enters its host cells where the iron is limited due to host sequestration. In particular, for root pathogens such as *C. victoriae*, sequestration may be stronger in the root surface. This could explain why the *cps1* mutant showed drastically reduced virulence. The FER1 could be required to release iron from the CPS1 siderophore which explains its location near the *CPS1* gene. Moreover, fungal strains could be cultured in iron-limiting conditions because CPS1, and likely other genes in the cluster maybe turned on only during conditions of iron depletion.

In a preferred embodiment, the polypeptides, including those having substantially similar activities to SEQ ID NO:47, SEQ ID NO:49, or SEQ ID NO:56 are encoded by nucleotide sequences derived from fungi, preferably from pathogenic fungi, desirably identical or substantially similar to the nucleotide sequences set forth in SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55 or the complement thereof.

In another preferred embodiment, the present invention describes a method for identifying agents having the ability to inhibit or reduce the activity of any one or more of SEQ ID NO:47, SEQ ID NO:49 or SEQ ID NO:56 in fungi. Preferably, a transgenic "knockout" fungus and/or fungal cell, is obtained which preferably is stably transformed, which comprises a deletion in any of

SEQ ID NO:46, SEQ ID NO:48 or SEQ ID NO:55. Thus, in one embodiment, the gene product encoded by the nucleotide sequence is not expressed, or has reduced or aberrant expression. In another embodiment, the transgenic fungus or cell comprises the corresponding non-deleted sequences linked to a promoter to
5 yield a gene product which is overexpressed. An agent is then contacted with the transgenic fungus and/or cell, and the growth development, virulence or pathogenicity of the transgenic fungus and/or cell is determined relative to the growth, development, or pathogenicity, of the corresponding transgenic fungus and/or cell to which the agent was not applied; or to the corresponding non-
10 transgenic fungus and/or cell.

The present invention generally relates to an isolated nucleic acid molecule from a fungal pathogen encoding a CPS1 peptide synthetase, an iron reductase or a permease/MFS transporter. In a preferred embodiment, a DNA molecule has a nucleotide sequence which hybridizes to a DNA molecule having
15 a sequence corresponding to SEQ ID NO:46, SEQ ID NO:48 or SEQ ID NO:55. Other DNA molecules of the present invention include DNA molecules that have a sequence which is greater than 65% identical to the nucleotide sequence of SEQ ID NO:46, SEQ ID NO: 48 or SEQ ID NO:55. Nucleotide sequence similarity is determined by the BLAST program with the default parameters
20 (Altschul et al., "Basic Local Alignment Search Tool," *J. Mol. Biol.*, 215:403 (1990). Preferred sequences include those DNA molecules which will hybridize to a nucleic acid molecule having the sequence of SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55 or the complement thereof. Preferably, the DNA molecules hybridize to SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:55, or its
25 complement under low or moderate, or stringent conditions.

Other proteins or polypeptides of the present invention include polypeptides having an amino acid sequence which has at least 75% similarity to the amino acid sequence of SEQ ID NO:47, SEQ ID NO:49 or SEQ ID NO:56. In a preferred embodiment of the invention, the protein or polypeptide will have
30 at least 90% similarity with SEQ ID NO:47, SEQ ID NO:49 or SEQ ID NO:56.

In addition, the nucleic acid molecules of the invention may be modified, adapted, and optimized in such a manner that, when transferred into an appropriate host cell, the modified polynucleotide confers an altered phenotype brought about by the polypeptide encoded by the modified sequence. One
5 advantage of this method is that it can be used to rapidly evolve any protein without knowledge of its structure. Peptide synthetase, iron reductase and/or permease/MFS transporter polynucleotides can be altered using sequence-shuffling methods as described by WO 00/28008 and references therein. Peptide synthetases of the invention can be recombined with other peptide synthetases,
10 iron reductases and/or permeases/MFS transporters to generate peptide synthetases, iron reductases and/or permeases/MFS transporters of desired and/or novel specificity and/or activity, and thus generate desired and/or novel non-encoded peptide products. Such novel peptide synthetases, iron reductases and/or permeases/MFS transporters would have at least one active domain or
15 other desired property-imparting domain (e.g., binding, enzymatic activity, specificity determining).

Briefly, sequences or fragments of sequences are shuffled by various recombinatorial methods, the shuffled polynucleotide is introduced into a suitable host for expression, the resulting phenotype is measured and the
20 modified phenotype is compared with the phenotype produced by unmodified sequence. Here, "phenotype" refers to the trait of interest and may include measuring the amount, conformation, composition, or enzymatic activity of the polypeptide encoded, if the sequence shuffling is being performed, to modify a single protein. Phenotype may also be assessed by measuring the effect of
25 expression of the modified peptide synthetase, iron reductase and/or permease/MFS transporter polynucleotide on expression of other genes, on cellular processes such as respiration or glycolysis, on tissue-level processes such as cell shape and size, and on organismal traits such as pathogenicity and/or virulence. Sequence-shuffled peptide synthetase polynucleotides producing a
30 desirable phenotype are then selected, further modified, and the resulting phenotype is measured. The shuffling and selection process is performed

iteratively until sequence shuffled polynucleotides encoding at least one polypeptide producing the desired phenotype is obtained, or until optimization of the trait of interest has plateaued and no further improvement is seen in subsequent rounds of shuffling and selection. Alternately, multiple rounds of recombination of peptide synthetase sequences may be performed prior to any selection step, with the aim of increasing the diversity of resulting populations nucleic acids prior to selection.

At least five general classes of recombination methods may be applied to peptide synthetase, iron reductase and/or permease/MFS transporter polynucleotides. First, the nucleic acids of peptide synthetase, iron reductase and/or permease/MFS transporter polynucleotides can be recombined *in vitro* by any of a variety of techniques including DNase digestion of polynucleotides followed by ligation and/or PCR reassembly of the polynucleotides. Second, polynucleotides can be recursively recombined *in vivo*, for example by allowing recombination to occur between an introduced peptide synthetase, iron reductase and/or permease/MFS transporter polynucleotide and homologous sequences in a cell. Third, whole cell genome recombination methods can be used in which whole genomes of cells are recombined, optionally including spiking the genomic (nuclear and/or plastid) recombination mixtures with the peptide synthetase, iron reductase and/or permease/MFS transporter sequences of interest. Fourth, synthetic recombination methods can be used, in which oligonucleotides corresponding to different homologs of the peptide synthetase, iron reductase and/or permease/MFS transporter sequence are synthesized and reassembled in PCR or ligation reactions which also include oligonucleotides which correspond to more than one allelic variant, thereby generating new recombined polynucleotides. Fifth, *in silico* methods of recombination can be carried out in which genetic algorithms are used in a computer to recombine sequence strings which correspond to homologs of the peptide synthetase sequences of interest. The resulting recombined sequence strings are optionally converted into nucleic acids by synthesis of nucleic acids which correspond to the recombined sequences. Such synthesis could proceed by oligonucleotide

synthesis and gene reassembly techniques. Any of the preceding general recombination formats can be practiced reiteratively to generate a more diverse set of recombinant nucleic acids.

5 The ever-increasing quantity and quality of data being accumulated not only about gene sequence, structure and function, but also about gene expression patterns and proteins interactions on genomic scales, makes it no longer feasible to deal with genetic data on an item-by-item basis but instead, necessary to create new ways of discovering biological information by *in silico* data mining. "Data mining" as used herein, refers to exploration and analysis of large quantities of data, by automatic and semi-automatic means, in order to discover meaningful patterns and rules. Data mining is applied to molecular sequence and structure data, gene expression and other high-throughput data, and to existing knowledge in the scientific literature, including making meaningful connections between different forms of knowledge and data.

15 A variety of data mining tools can be applied using the peptide synthetase, iron reductase and/or permease/MFS transporter sequences of the present invention. A method appropriate for use in sequence databases which contain long stretches of data known as long-pattern data sets, is that disclosed in U.S. Patent No. 6,138,117, which uses a look-ahead scheme for quickly
20 identifying long patterns that is not limited to the initialization phase, an heuristic item-ordering policy for tightly focusing the search, and a support-lower-bounding scheme that is also applicable to other algorithms. Recursive partitioning is useful to elucidate structure-activity relations and to guide decision-making for high-throughput screening of compounds for their effects on
25 peptide synthetase polypeptides, for example as described by Hertzog et al. (J. Pharmacol Toxicol Methods 42:207 (1999)) for sequential screening of G-protein-coupled receptors. The peptide synthetase, iron reductase and/or permease/MFS transporter sequences of the present invention may be applied to digital differential display (DDD) to analyze differential expression and create an
30 electronic expression profile for a variety of physiological conditions. Peptide synthetase, iron reductase and/or permease/MFS transporter sequence data can

be analyzed to predict protein domains using the BLAST algorithm. Higher-order correlations among peptide synthetase, iron reductase and/or permease/MFS transporter proteins may be predicted by using peptide synthetase protein sequence data to compare sets of sequence-distant sites displaying high mutual information which may bespeak important structural or functional features, a methodology that overcomes the limitations of previous methods which examined only single-residue features or pairwise interactions. (Steeg et al., Pac Symp Biocomput 1998:573 (1998)).

Peptide synthetase, iron reductase and/or permease/MFS transporter polypeptide sequences having structures expressed in a computer-readable form can be evaluated for function using functional site descriptors (FSDs) for a biomolecule functional site having a specific biological function, as described in the publication WO 00/11206. FSDs can be used to identify or screen for a novel function in one or more peptide synthetase, iron reductase and/or permease/MFS transporter polypeptides, to confirm a previously identified or suspected function of a protein, to evaluation the effects of sequence shuffling on protein function, or to provide further information about a specific functional site in a peptide synthetase, iron reductase and/or permease/MFS transporter polypeptide.

FSDs are geometric representations of protein functional sites, typically defining spatial configurations of functional sites by providing a three-dimensional (3D) representation of a protein functional site. Preferred functional sites represented by FSDs include a ligand binding domain, an ion or cofactor binding site, a site or domain for protein-protein interaction, or an enzymatic active site. An FSD typically comprises a set of geometric constraints for one or more atoms in each of two or more amino acid residues comprising a function site of a protein. Geometric constraints of an FSD may comprise an atomic position specified by a set of 3D coordinates, an interatomic distance, an interatomic bond angle, or conformational constraints imposed by residues at a site or by secondary structure such as a zinc finger, leucine zipper, helix, or α strand, where these constraints may be expressed either as fixed coordinates or

ranges. Libraries of FSDs can comprise at least two FSDs for at least one of the biological functions represented by the library.

FSDs are used to probe protein structures to determine if such structures contain the functional sites described by the corresponding FSDs. Peptide
5 synthetase, iron reductase and/or permease/MFS transporter polypeptides to be screened can comprise an unmodified sequence selected from SEQ ID NO:47, SEQ ID NO:49 or SEQ ID NO:56, or a modified form derived from random or directed sequence shuffling as previously described. Typically, functional screening methods comprise applying a FSD to a structure of a peptide
10 synthetase, iron reductase and/or permease/MFS transporter polypeptide, where the structure may be determined by x-ray crystallography, nuclear magnetic resonance, by a computer “*ab initio*” folding program a homology program, or a “threading” program, and expressed in a computer-readable form.

The function of a peptide synthetase, iron reductase and/or
15 permease/MFS transporter polypeptide whose structure is expressed in computer-readable form can be screened by applying an FSD to the structure of a peptide synthetase, iron reductase and/or permease/MFS transporter polypeptide and determining whether the peptide synthetase, iron reductase and/or permease/MFS transporter polypeptide structure matches, or satisfies, the
20 constraints of the FSD. Libraries of FSDs can be used to probe for or evaluate the activity or function associated with the FSD in one or more protein structures.

The DNA molecule encoding the CPSI, iron reductase polypeptide and/or permease/MFS transporter of the present invention can be incorporated in cells
25 using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e., not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary
30 elements for the transcription and translation of the inserted protein-coding sequences. U.S. Patent No. 4,237,224, describes the production of expression

systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in culture. Recombinant
5 genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gtWEST.B, Charon 4, and plasmid
10 vectors such as pBR22, pBR325, pACYC177, pACYC184, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif), pQE, pIH821, pGEX, pET series (see Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression
15 Technology, vol.185 (1990)), and any derivatives thereof. Suitable vectors are continually being developed and identified. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Maniatis et al. or
20 Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1982 or 1989, respectively).

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following:
25 bacteria transformed with bacteriophage DNA, plasmid DNA) or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria or transformed via particle bombardment (i.e., biolistics). The expression elements
30 of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and

translation elements can be used. Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA, "mRNA" translation). Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of DNA in procaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of DNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S, rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Koberts and Lauer, Methods in Enzymology 68:473 (1979).

Promoters vary in their "strength" (i.e., their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*; its bacteriophages, or plasmids, promoters such as the phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the PR and PL promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (tac) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the insert

gene. Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operons, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the lac operon is induced by the addition of lactose or IPTG (isopropylthiobeta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls. Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires a Shine-Dalgarno ("SD" sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the N gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used. The present invention also relates to anti-sense nucleic acid for essential cell proteins, such as replication proteins which serve to tender host cells incapable of further cell growth and division. Anti-sense regulation has been described by Rosenberg et al., Nature, 313:703 (1985); Preiss et al., Nature, 313:27 (1985); Melton, Proc. Natl. Acad. Sci. USA, 82:144 (1985); Izaut et al., Science, 229:342 (1985); Kim et al., Cell, 42:129 (1985); Bestka et al., Proc Natl.. Acad. Sci. USA, 81:7525 (1984); Coleman et al., Cell, 37:429 (1984); and McQany et al., Proc. Natl. Acad. Sci. USA, 83:399 (1986), which are hereby incorporated by reference.

Once the isolated DNA molecules encoding the CPS1 polypeptide or iron reductase have been cloned into an expression system, they are ready to be incorporated into a host cell. Such incorporation can be carried out by the

various forms of transformation noted above, depending upon the vector host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like. In the present invention, the host cells are from plants such as corn, oat, grass, weeds, bamboo, and
5 sugarcane. In this aspect of the present invention, large numbers of compounds can be screened for their activity as inhibitors of CPS1 protein, iron reductase or permease/MFS transporter by a high throughput screening assay as described in U.S. Patent No. 5,767,946. Generally, a library of compounds is assayed for inhibition of an enzyme catalyzed reaction and the amounts of fluorescence
10 bound to individual suspendable solid supports measured to determine the degree of inhibition. For example, the amount of fluorescence bound to a microbead in the presence of inhibitory compounds is greater than for non-inhibitory compounds. The amounts of fluorescence bound to individual beads are determined by confocal microscopy. Using this type of assay, inhibition can be
15 determined, e.g., of a peptide synthetase such as CPS1. For CPS1 the substrate can be amino acids (or hydroxy acids), linked at one end to the microbead and at the other end to a fluorescent label. The enzyme inhibitors can be utilized to impart fungal resistance to a variety of vertebrate organisms.

Another aspect of the present invention involves using one or more of the
20 above DNA molecules encoding the CPS1 polypeptide or a gene encoding an enzyme that degrades the CPS1 product to transform organisms to impart fungal resistance to the organism. This concept of pathogen-derived resistance, according to U.S. Patent No. 5,840,481 is that host resistance to a particular parasite can effectively be engineered by introducing a gene, gene fragment, or
25 modified gene or gene fragment of the pathogen into the host. This approach is based on the fact that in any parasite-host interaction, there are certain parasite-encoded cellular functions (activities) that are essential to the parasite but not to the host and that when one of the essential functions of the parasite such as survival or reproduction is disrupted, the parasitic process will be stopped.
30 "Disruption" refers to any change that diminishes the survival, reproduction, or ineffectivity of the parasite. Such essential functions, which are under the

control of the parasite's genes, can be disrupted by the presence of a corresponding gene product in the host which is (1) dysfunctional, (2) in excess, or (3) appears in the wrong context or at the wrong developmental stage in the parasite's life cycle. If such faulty signals are designed specifically for parasitic cell functions, they will have little effect on the host. Therefore, the procedure for making organisms, for example, resistant to infection by one or more fungus involve isolating DNA coding for a gene such as CPS1 of a fungus, operably linking the DNA within an expression vector; and transforming a cell or tissue with the expression vector. The transformed cells or tissue in the presence of the fungus such as *Cochliobolus heterostrophus* where the CPS1 DNA is expressed as a gene product and the CPS protein disrupts the essential activity of the fungi.

Dosages, Formulations and Routes of Administration of the Agents of the Invention

The therapeutic agents identified by the methods of the invention may be administered at dosages of at least about 0.01 to about 100 mg/kg, more preferably about 0.1 to about 50 mg/kg, and even more preferably about 0.1 to about 30 mg/kg, of body weight, although other dosages may provide beneficial results. The amount administered will vary depending on various factors including, but not limited to, the agent chosen, the disease, whether prevention or treatment is to be achieved, and if the agent is modified for bioavailability and *in vivo* stability.

Administration of a sense or antisense nucleic acid molecule encoding a therapeutic agent may be accomplished through the introduction of cells transformed with an expression cassette comprising the nucleic acid molecule (see, for example, WO 93/02556) or the administration of the nucleic acid molecule (see, for example, Felgner et al., U.S. Patent No. 5,580,859, Pardoll et al., Immunity, 3:165 (1995); Stevenson et al., Immunol. Rev., 145:211 (1995); Molling, J. Mol. Med., 75:242 (1997); Donnelly et al., Ann. N.Y. Acad. Sci., 772:40 (1995); Yang et al., Mol. Med. Today, 2:476 (1996); Abdallah et al., Biol. Cell, 85:1 (1995)). Pharmaceutical formulations, dosages and routes of

administration for nucleic acids are generally disclosed, for example, in Felgner et al., *supra*.

The therapeutic agents of the invention are amenable to chronic use for prophylactic purposes, preferably by systemic administration.

5 Administration of the therapeutic agents in accordance with the present invention may be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the agents of the invention may be essentially continuous over
10 a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated.

One or more suitable unit dosage forms comprising the therapeutic agents of the invention, which, as discussed below, may optionally be formulated for sustained release, can be administered by a variety of routes including oral, or
15 parenteral, including by rectal, buccal, vaginal and sublingual, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, intrathoracic, intrapulmonary and intranasal routes. The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to pharmacy. Such methods may include the
20 step of bringing into association the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

When the therapeutic agents of the invention are prepared for oral
25 administration, they are preferably combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. The total active ingredients in such formulations comprise from 0.1 to 99.9% by weight of the formulation. By "pharmaceutically acceptable" it is meant the carrier, diluent, excipient, and/or salt must be compatible with the
30 other ingredients of the formulation, and not deleterious to the recipient thereof. The active ingredient for oral administration may be present as a powder or as

granules; as a solution, a suspension or an emulsion; or in achievable base such as a synthetic resin for ingestion of the active ingredients from a chewing gum. The active ingredient may also be presented as a bolus, electuary or paste.

Formulations suitable for vaginal administration may be presented as
5 pessaries, tampons, creams, gels, pastes, douches, lubricants, foams or sprays containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate. Formulations suitable for rectal administration may be presented as suppositories.

Pharmaceutical formulations containing the therapeutic agents of the
10 invention can be prepared by procedures known in the art using well-known and readily available ingredients. For example, the agent can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following fillers and
15 extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose, HPMC and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption
20 accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

For example, tablets or caplets containing the agents of the invention can
25 include buffering agents such as calcium carbonate, magnesium oxide and magnesium carbonate. Caplets and tablets can also include inactive ingredients such as cellulose, pregelatinized starch, silicon dioxide, hydroxy propyl methyl cellulose, magnesium stearate, microcrystalline cellulose, starch, talc, titanium dioxide, benzoic acid, citric acid, corn starch, mineral oil, polypropylene glycol,
30 sodium phosphate, and zinc stearate, and the like. Hard or soft gelatin capsules containing an agent of the invention can contain inactive ingredients such as

gelatin, microcrystalline cellulose, sodium lauryl sulfate, starch, talc, and titanium dioxide, and the like, as well as liquid vehicles such as polyethylene glycols (PEGs) and vegetable oil. Moreover, enteric coated caplets or tablets of an agent of the invention are designed to resist disintegration in the stomach and
5 dissolve in the more neutral to alkaline environment of the duodenum.

The therapeutic agents of the invention can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes.

10 The pharmaceutical formulations of the therapeutic agents of the invention can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an emulsion or suspension.

Thus, the therapeutic agent may be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous
15 infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The active ingredients may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively,
20 the active ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

These formulations can contain pharmaceutically acceptable vehicles and adjuvants which are well known in the prior art. It is possible, for example, to
25 prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint, chosen, in addition to water, from solvents such as acetone, ethanol, isopropyl alcohol, glycol ethers such as the products sold under the name "Dowanol", polyglycols and polyethylene glycols, C₁-C₄ alkyl esters of short-chain acids, preferably ethyl or isopropyl lactate, fatty acid
30 triglycerides such as the products marketed under the name "Miglyol", isopropyl myristate, animal, mineral and vegetable oils and polysiloxanes.

The compositions according to the invention can also contain thickening agents such as cellulose and/or cellulose derivatives. They can also contain gums such as xanthan, guar or carbo gum or gum arabic, or alternatively polyethylene glycols, bentones and montmorillonites, and the like.

5 It is possible to add, if necessary, an adjuvant chosen from antioxidants, surfactants, other preservatives, film-forming, keratolytic or comedolytic agents, perfumes and colorings. Also, other active ingredients may be added, whether for the conditions described or some other condition.

 For example, among antioxidants, t-butylhydroquinone, butylated
10 hydroxyanisole, butylated hydroxytoluene and α -tocopherol and its derivatives may be mentioned. The galenical forms chiefly conditioned for topical application take the form of creams, milks, gels, dispersion or microemulsions, lotions thickened to a greater or lesser extent, impregnated pads, ointments or sticks, or alternatively the form of aerosol formulations in spray or foam form or
15 alternatively in the form of a cake of soap.

 Additionally, the agents are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal or respiratory tract, possibly over a period of time. The coatings,
20 envelopes, and protective matrices may be made, for example, from polymeric substances, such as polylactide-glycolates, liposomes, microemulsions, microparticles, nanoparticles, or waxes. These coatings, envelopes, and protective matrices are useful to coat indwelling devices, e.g., stents, catheters, peritoneal dialysis tubing, and the like.

25 The therapeutic agents of the invention can be delivered via patches for transdermal administration. See U.S. Patent No. 5,560,922 for examples of patches suitable for transdermal delivery of a therapeutic agent. Patches for transdermal delivery can comprise a backing layer and a polymer matrix which has dispersed or dissolved therein a therapeutic agent, along with one or more
30 skin permeation enhancers. The backing layer can be made of any suitable material which is impermeable to the therapeutic agent. The backing layer

serves as a protective cover for the matrix layer and provides also a support function. The backing can be formed so that it is essentially the same size layer as the polymer matrix or it can be of larger dimension so that it can extend beyond the side of the polymer matrix or overlay the side or sides of the polymer matrix and then can extend outwardly in a manner that the surface of the extension of the backing layer can be the base for an adhesive means. Alternatively, the polymer matrix can contain, or be formulated of, an adhesive polymer, such as polyacrylate or acrylate/vinyl acetate copolymer. For long-term applications it might be desirable to use microporous and/or breathable backing laminates, so hydration or maceration of the skin can be minimized.

Examples of materials suitable for making the backing layer are films of high and low density polyethylene, polypropylene, polyurethane, polyvinylchloride, polyesters such as poly(ethylene phthalate), metal foils, metal foil laminates of such suitable polymer films, and the like. Preferably, the materials used for the backing layer are laminates of such polymer films with a metal foil such as aluminum foil. In such laminates, a polymer film of the laminate will usually be in contact with the adhesive polymer matrix.

The backing layer can be any appropriate thickness which will provide the desired protective and support functions. A suitable thickness will be from about 10 to about 200 microns.

Generally, those polymers used to form the biologically acceptable adhesive polymer layer are those capable of forming shaped bodies, thin walls or coatings through which therapeutic agents can pass at a controlled rate. Suitable polymers are biologically and pharmaceutically compatible, nonallergenic and insoluble in and compatible with body fluids or tissues with which the device is contacted. The use of soluble polymers is to be avoided since dissolution or erosion of the matrix by skin moisture would affect the release rate of the therapeutic agents as well as the capability of the dosage unit to remain in place for convenience of removal.

Exemplary materials for fabricating the adhesive polymer layer include polyethylene, polypropylene, polyurethane, ethylene/propylene copolymers,

ethylene/ethylacrylate copolymers, ethylene/vinyl acetate copolymers, silicone elastomers, especially the medical-grade polydimethylsiloxanes, neoprene rubber, polyisobutylene, polyacrylates, chlorinated polyethylene, polyvinyl chloride, vinyl chloride-vinyl acetate copolymer, crosslinked polymethacrylate polymers (hydrogel), polyvinylidene chloride, poly(ethylene terephthalate), butyl rubber, epichlorohydrin rubbers, ethylenvinyl alcohol copolymers, ethylene-vinyloxyethanol copolymers; silicone copolymers, for example, polysiloxane-polycarbonate copolymers, polysiloxanepolyethylene oxide copolymers, polysiloxane-polymethacrylate copolymers, polysiloxane-alkylene copolymers (e.g., polysiloxane-ethylene copolymers), polysiloxane-alkylenesilane copolymers (e.g., polysiloxane-ethylenesilane copolymers), and the like; cellulose polymers, for example methyl or ethyl cellulose, hydroxy propyl methyl cellulose, and cellulose esters; polycarbonates; polytetrafluoroethylene; and the like.

Preferably, a biologically acceptable adhesive polymer matrix should be selected from polymers with glass transition temperatures below room temperature. The polymer may, but need not necessarily, have a degree of crystallinity at room temperature. Cross-linking monomeric units or sites can be incorporated into such polymers. For example, cross-linking monomers can be incorporated into polyacrylate polymers, which provide sites for cross-linking the matrix after dispersing the therapeutic agent into the polymer. Known cross-linking monomers for polyacrylate polymers include polymethacrylic esters of polyols such as butylene diacrylate and dimethacrylate, trimethylol propane trimethacrylate and the like. Other monomers which provide such sites include allyl acrylate, allyl methacrylate, diallyl maleate and the like.

Preferably, a plasticizer and/or humectant is dispersed within the adhesive polymer matrix. Water-soluble polyols are generally suitable for this purpose. Incorporation of a humectant in the formulation allows the dosage unit to absorb moisture on the surface of skin which in turn helps to reduce skin irritation and to prevent the adhesive polymer layer of the delivery system from failing.

Therapeutic agents released from a transdermal delivery system must be capable of penetrating each layer of skin. In order to increase the rate of permeation of a therapeutic agent, a transdermal drug delivery system must be able in particular to increase the permeability of the outermost layer of skin, the stratum corneum, which provides the most resistance to the penetration of molecules. The fabrication of patches for transdermal delivery of therapeutic agents is well known to the art.

For administration to the upper (nasal) or lower respiratory tract by inhalation, the therapeutic agents of the invention are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of the therapeutic agent and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatine or blister packs from which the powder may be administered with the aid of an inhalator, insufflator or a metered-dose inhaler.

For intra-nasal administration, the therapeutic agent may be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker).

The local delivery of the therapeutic agents of the invention can also be by a variety of techniques which administer the agent at or near the site of disease. Examples of site-specific or targeted local delivery techniques are not intended to be limiting but to be illustrative of the techniques available. Examples include local delivery catheters, such as an infusion or indwelling

catheter, e.g., a needle infusion catheter, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct applications.

For topical administration, the therapeutic agents may be formulated as is known in the art for direct application to a target area. Conventional forms for this purpose include wound dressings, coated bandages or other polymer coverings, ointments, creams, lotions, pastes, jellies, sprays, and aerosols, as well as in toothpaste and mouthwash, or by other suitable forms, e.g., via a coated condom. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The active ingredients can also be delivered via iontophoresis, e.g., as disclosed in U.S. Patent Nos. 4,140,122; 4,383,529; or 4,051,842. The percent by weight of a therapeutic agent of the invention present in a topical formulation will depend on various factors, but generally will be from 0.01% to 95% of the total weight of the formulation, and typically 0.1-25% by weight.

When desired, the above-described formulations can be adapted to give sustained release of the active ingredient employed, e.g., by combination with certain hydrophilic polymer matrices, e.g., comprising natural gels, synthetic polymer gels or mixtures thereof.

Drops, such as eye drops or nose drops, may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs. Drops can be delivered via a simple eye dropper-capped bottle, or via a plastic bottle adapted to deliver liquid contents dropwise, via a specially shaped closure.

The therapeutic agent may further be formulated for topical administration in the mouth or throat. For example, the active ingredients may be formulated as a lozenge further comprising a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the composition in an inert base

such as gelatin and glycerin or sucrose and acacia; mouthwashes comprising the composition of the present invention in a suitable liquid carrier; and pastes and gels, e.g., toothpastes or gels, comprising the composition of the invention.

5 The formulations and compositions described herein may also contain other ingredients such as antimicrobial agents, or preservatives. Furthermore, the active ingredients may also be used in combination with other therapeutic agents, for example, oral contraceptives, bronchodilators, anti-viral agents, steroids and the like.

10 The invention will be further described by the following non-limiting examples.

15

Example 1

Mutant Preparation and Characterization

Materials and Methods

Strains, Media, Crosses and Transformation. C4 (*ToxI*⁺; MAT-2) and C5 (*ToxI*⁻; MAT-1) are members of near-isogenic *C. heterostrophus* strains (Leach et al., 1982, *supra*). R.C4.2696 (*Tox*⁺; MAT-2; *hygB*^R) is a C4-derived mutant generated using the REMI mutagenesis procedure (Lu et al., Proc. Natl. Acad. Sci. USA, 91:12649 (1994)). Strains 1301R33 (*Tox*⁻; MAT-2; *hygB*^R), 1301R45 (*Tox*⁻; MAT-1; *hygB*^R) 1301R26 (*Tox*⁺; MAT-2; *hygB*^R) are progeny of the cross C5 X R.C4.2696. Culture media, including CM (complete medium), CMX (complete medium with xylose instead of glucose), CMNS (CM with salts omitted), and MM (minimal medium) have been described, as have mating procedures (Leach et al., 1982, *supra*; Turgeon et al., Mol. Gen. Genet., 201:450 (1985)). All strains were grown at 24°C under the warm white light or black light (F40/350BL) (Sylvania Inc., Danvers, MA). Ascospore germination was done at 32°C in the dark for 3 days. REMI transformants were purified by transferring the transformants from the original REMI plates to fresh CMNS

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medium containing hygromycin B (Calbiochem^R) at 80 µg/ml. For conidiation, stable transformants were transferred to CMX containing the same drug but at a higher concentration (120 µg/ml) to compensate for reduced drug activity due to the inhibition by the salts in the medium. Single conidia were picked up under a dissecting microscope and grown on CMNS hygromycin B plates; stable colonies were then transferred to individual CMX/hygromycin plates. All purified transformants were stored at -70°C in CM liquid medium containing 25% of glycerol in 96-well microtiter dishes.

Bioassays. Fungal strains were grown on CMX plates (100 X 15 mm) for 7-10 days at 24°C under the light for maximum conidiation. To verify normal T-toxin production by a race T isolate, 1.0 ml of T-toxin-sensitive *E. coli* (DH5a) cells were evenly spread on LB medium containing ampicillin (100 µg/ml) and the plates were allowed to air dry for 30 minutes in a laminar hood. Agar plugs bearing fungal mycelia were inoculated (upside down) onto the *E. coli* cell lawn and the plates were incubated at 32°C. Wild type race T and race O were used as controls for each assay plate. T-toxin-producing strains of the fungus will inhibit growth of the *E. coli* cells and produce halos. *Tox*⁻ mutants can be distinguished from wild type by failure to produce a halo (tight) or by production of halos smaller (leaky) or larger than wild type (overproducing). All *Tox*⁻ mutants were transferred to Fries medium (Pringle et al., Phytopathology, 47:369 (1957)), which optimizes toxin production, and retested.

T-cytoplasm corn plants (inbred W64A) are used to verify the *Tox*⁻ mutants identified from the *E. coli* assay using the procedure described below. Mutants defective in T-toxin production fail to produce typical race T symptoms on T-corn. Pathogenicity phenotype on N-cytoplasm corn and virulence of *Tox*⁺ strains to T-cytoplasm corn were determined by a plant assay where, about 3,000 transformants generated using the REMI mutagenesis procedure (Lu et al., Proc. Natl. Acad. Sci. USA, 91:12649 (1994)) were screened for mutants defective in ability to cause disease on corn plants. Two week old N-cytoplasm corn plants (inbred W64A) grown in the green house (5-6 plants in one 4" X 6" pot) were inoculated with 5 ml conidial suspensions (10⁵ conidia/ml) using a pressurized

Preval Spray Gun Power Unit thin layer chromatography sprayer (Alltech Associates, Deerfield, IL), incubated in the mist chamber for 24 hours (23 °C) and then taken to the growth chamber (23 °C, 80% humidity, 14 hours of light). The mutant phenotypes were determined by occurrence of apparent variations in disease symptom development, mainly by lesion size comparison. Mutants producing lesions smaller than wild type were retested and lengths of typical lesions from each mutant were compared with wild type 7 days after inoculation and measurements were taken for statistical evaluation.

DNA manipulations and sequencing. Genomic and plasmid DNA preparation, restriction enzyme digestions, gel electrophoresis and gel blot analysis were done using standard protocols (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press (1989)). DNA was sequenced at the Cornell DNA Sequencing Facility using TaqCycle automated sequencing with DyeDeoxy terminators (Applied Biosystems, Foster City, CA). pUCATPH was used for subcloning (Table 1). Primers used for sequencing (Table 2) were designed using Primer Select (DNASTAR Inc., LaserGene System) and synthesized by the Cornell Oligonucleotide Synthesis Facility. Sequencing of each plasmid clone was initiated with vector-specific primers or primers designed to previously determined sequences. Sequences obtained were analyzed using the same system and nucleotide or protein database searches were performed with the BLAST program (Altschul et al., J. Mol. Biol., 215:403 (1990)).

25

Table 1. Transformation vectors and clones used.

| Plasmid | Length (kb) ^a | Characteristics (See U.S. application Serial Nos. 60/252,649 and 60/252,732) |
|----------|--------------------------|--|
| pUCATPH | 5.1 | See Figure 14 in U.S. application Serial No. 60/252,649. |
| PUCATPHN | 4.6 | Cloning vector, same as pUCATPH but lacking a 420 bp <i>NarI</i> fragment containing the <i>HindIII</i> site |
| p214B7 | <u>9.2</u> | A clone containing pUCATPH recovered from the tagged site in mutant R.C4.2696 by religation of <i>BglIII</i> -digested genomic DNA |
| p214M1 | <u>6.3</u> | As above but with <i>MscI</i> -digested genomic DNA |
| p214S1 | <u>9.3</u> | As above but with <i>SacI</i> -digested genomic DNA |
| p214S1N | <u>3.3</u> | <i>NarI</i> fragment derived from 214S1 containing a 0.8 kb <i>NarI</i> - <i>SacI</i> fragment of genomic DNA ligated to pUC18 |
| p214SNP | <u>8.4</u> | Vector for targeted integration constructed by ligating <i>HindIII</i> -digested pUCATPH into the <i>HindIII</i> site of p214S1N |
| p118BSP | <u>7.3</u> | Vector for targeted integration constructed by ligation of a 2.2 kb <i>SacI</i> fragment of p118BC4 into the <i>SacI</i> site of pUCATPH |
| p118BCS | <u>5.4</u> | Vector for targeted integration constructed by ligation of a 0.8 kb <i>SspI</i> fragment of p118BC4 into the <i>SspI</i> site of pUCATPHN |
| p118B14 | <u>10.4</u> | A clone recovered from the p214SNP integration site in transformant #f118 by ligation of a <i>BglIII</i> -digested genomic DNA fragment containing the entire vector |
| p118BC4 | <u>6.7</u> | A clone recovered from same site as above but by ligation of a <i>BclI</i> -digested genomic DNA fragment containing part of vector (214SNP) sequence |

| Plasmid | Length (kb) ^a | Characteristics (See U.S. application Serial Nos. 60/252,649 and 60/252,732) |
|---------|--------------------------|--|
| p9P2 | <u>7.3</u> | A clone recovered from the p118BSP integration site in transformant #9 by ligation of a <i>Pst</i> I-digested genomic DNA fragment containing pUC18 |
| p12H6 | <u>8.0</u> | A clone recovered from the p118BCS integration site in transformant #12 by ligation of a <i>Hind</i> III-digested genomic DNA fragment containing the entire vector. |

^a An underlined kb number indicates that the plasmid carries genomic DNA sequences.

- 5 Table 2. Primers used for sequencing recovered genomic DNA flanking the REMI insertion site at the R.C4 2696 mutation.

| Name ^a | Position ^b | Sequence ^c | Plasmid ^d | Origin ^e |
|-------------------|-----------------------|-----------------------|----------------------|---------------------|
| M13RMT | | SEQ ID NO:4 | A | pUC18 |
| 1. RP1b | 775 | SEQ ID NO:5 | A | 214B7TrpC |
| 2. RP2 | 604 | SEQ ID NO:6 | A | 214B7RP1b |
| 3. RP3 | 119 | SEQ ID NO:7 | A | 214B7RP2 |
| 4. RP4 | -232 | SEQ ID NO:8 | A | 214B7RP3 |
| 5. RP5 | -812 | SEQ ID NO:9 | A | 214B7RP4 |
| 6. RP5b | -1215 | SEQ ID NO:10 | A | 214B7RP4 |
| 7. RP6 | -1392 | SEQ ID NO:11 | A | 214B7RP5 |
| 8. RP7 | -1839 | SEQ ID NO:12 | A | 214B7RP6 |
| TrpC | | SEQ ID NO:13 | A | PUCATPH |
| 9. FP1 | 1885 | SEQ ID NO:14 | A | 214B7TrpC |
| 10. FP1b | 1828 | SEQ ID NO:15 | B | 214B7TrpC |
| 11. FP2 | 2028 | SEQ ID NO:16 | B | 214M1FP1b |
| 12. FP3 | 2490 | SEQ ID NO:17 | C | 214M1FP2 |
| 13. FP4 | 2949 | SEQ ID NO:18 | C | 214S1FP3 |
| 14. FP4B | 2745 | SEQ ID NO:19 | C | 214S1FP4 |
| 15. FP5 | 3421 | SEQ ID NO:20 | C | 214S1FP4 |
| 16. FP6 | 3948 | SEQ ID NO:21 | C | 214S1FP5 |
| 17. FP7 | 4411 | SEQ ID NO:22 | C, D | 214S1FP6 |
| 18. FP8 | 5035 | SEQ ID NO:23 | D | 118B14FP7 |

| Name ^a | Position ^b | Sequence ^c | Plasmid ^d | Origin ^e |
|-------------------|-----------------------|-----------------------|----------------------|---------------------|
| 19. FP9 | 5457 | SEQ ID NO:24 | | 118BC4FP8 |
| 20. RP48 | 2865 | SEQ ID NO:25 | D | 214S1FP6 |
| 21. FP10 | 5790 | SEQ ID NO:26 | F | 9P2FP9 |
| 22. FP11 | 6327 | SEQ ID NO:27 | F | 9P2FP10 |
| 23. FP11b | 6211 | SEQ ID NO:28 | F | 9P2FP10 |
| 24. FP12 | 6457 | SEQ ID NO:29 | F | 9P2FP11 |
| 25. FP13 | 6854 | SEQ ID NO:30 | F | 9P2FP12 |
| 26. FP14 | 7400 | SEQ ID NO:31 | F | 9P2FP13 |
| 27. FP15 | 7771 | SEQ ID NO:32 | F | 9P2FP14 |
| 28. FP16 | 8145 | SEQ ID NO:33 | F | 9P2FP15 |
| 29. FP17 | 8492 | SEQ ID NO:34 | F | 9P2FP16 |
| M13F40 | | SEQ ID NO:35 | G | pUC18 |
| 30. RP1 | 8953 | SEQ ID NO:36 | G | 9P5M13F4 |
| 31. RP2 | 8559 | SEQ ID NO:37 | G | 9P5RP1 |

- ^a “RP” indicates reverse primer; “FP” indicates forward primer. Primers designed to genomic DNA sequences are numbered in order. Primers 1-17 have a leading number “214”; 18-20 with “118”; 21-29 with “9P2” and 30-31 with “9P5”. M13RMT (a M13R mutant version; there is a mutation in the polylinker of pUC18) and M13F-40 were provided by Cornell DNA Sequencing Facility. *TrpC* primer site is in the pUCATPH *TrpC* promoter region 38 bp from *SaII* site with sequencing direction from *SaII* to *KpnI*.
- ^b The position of the first base of each primer corresponds to the assembled sequence (*CPSI* + *TESI*, total 11.3 kb).
- ^c Each primer sequence is given in the 5' to 3' direction.
- ^d Plasmids used as templates for each sequencing reaction. A= p214B7; B=p214M1; C=p214S1; D=p118B14; E=p118BC4; F=p9P2; G=p9P5 (=9P2)
- ^e Original sequences that were used for primer design.

Results

Recovery of tagged DNA from the REMI insertion site and targeted gene disruption. Genomic DNA of mutant R.C4.2696 was digested with *BglIII*, *MscI* (no sites in pUCATPH) or *SacI* (which cuts the vector once) and purified by phenol extraction and ethanol precipitation, then dissolved in TE (pH 8.0). Ligation was performed in 50 µl reaction mixture, containing 1 x T4 DNA ligase buffer with 10 mM ATP, 60 units T4 DNA ligase (New England Biolabs, Beverly, MA) and 3 µg of *BglIII*-digested genomic DNA, at 14°C overnight. Ten µl of ligation mixture was used to transform 200 µl of competent DH5α cells,

prepared using the calcium chloride treatment (Sambrook et al., 1989, *supra*) to ampicillin resistance. Ampicillin resistant clones were analyzed by digestion of plasmid DNA with several diagnostic restriction enzymes and clones containing the REMI vector plus flanking genomic DNA were sequenced using the vector-specific primers (M13R or *TrpC*). Three plasmids, p214B7, p214M1 and p214S1 were recovered and used for sequencing. p214B7 contains 4.2 kb flanking DNA (3.4 left; 0.7 right); p214M1 contains 0.1 kb left flank that overlaps with p214B7 and 1.1 kb right flank that overlaps with p214S1, which contains 3.2 kb flanking DNA on the left only.

For targeted gene disruption in wild type, p214B7 was amplified and plasmid DNA purified by equilibrium centrifugation in CsCl-ethidium bromide gradients (Sambrook et al., 1989, *supra*). Thirty µg of plasmid DNA (linearized with *Bgl*III for double crossover integration) were used to transform wild type and the transformants were purified by isolation of single conidia, assayed for pathogenicity and characterized by gel blot analysis.

Sequence extension by targeted integration and plasmid rescue. Two overlapping cosmid clones were isolated by probing a genomic DNA library of C4 constructed on a cosmid vector, but both extended into the left region only of p214B7. To extend to the right, a chromosome walking strategy was employed. Three targeted gene disruption experiments (each followed by plasmid rescue) were done successively. In the first experiment, a vector was constructed as follows: p214S1 was digested with *Nar*I and religated to create p214S1N, which was then digested with *Hind*III and ligated into the *Hind*III site of pUCATPH to create p214SNP for transformation of race O (C5). One transformant (Tx118) resulting from homologous integration (confirmed by gel blot analysis) was used for plasmid rescue as described above. Two new plasmids p118B14 and p118BC4 were recovered, both of which carry sequence at the 3' end but only 172 and 680 bp more than p214S1, respectively. To continue the walk, p118B14 was digested with *Sac*I and ligated into the *Sac*I site of pUCATPH to create p118BSP. This vector was linearized with *Bgl*III and transformed into wild type and one plasmid, p9P2 was recovered (from transformant Tx9), which extends

4.4 kb into the region 3' of p118BC4 and contains the 3' end of *CPSI*. The recovered plasmid p9P2 includes the entire pUC18 sequence on p118BSP and 4.6 kb of genomic DNA that contains all of ORF1 (*CPSI*), including the stop codon (TAG) and 3.0 kb of genomic region 3' of the stop codon. A third
5 experiment was done in an attempt to recover a 15 kb *XhoI* fragment at the 3' end of that tagged gene. p118BCS was constructed by subcloning a 0.8 kb *SspI* fragment into the same site pUCATPHN. Plasmid rescue using *XhoI* digested-genomic DNA of a transformant (TXI2) failed to recover the 15 kb *XhoI* fragment, but p12H6 was recovered using *HindIII*-digested genomic DNA of the
10 same transformant; the genomic DNA matched that already cloned on p9P2.

Characterization of the REMI mutant. In all culture conditions used, mutant R.C4.2696 grew just like wild type with no variations in growth rate, color and morphological features. It produces normal appressorium-forming conidia that germinate and form infection structures like wild type when induced
15 on artificial surfaces and shows normal mating ability when crossed to wild type testers. No pleiotropic phenotypes associated with the mutation have been detected so far. The mutant differs from wild type in the ability to cause disease on corn plants.

The lengths of 100 typical lesions from corn leaves inoculated with wild
20 type race O and a mutant progeny R45 (*Tox*⁻, *hygB*^R) carrying the R.C4.2696 mutation were measured 7 days after inoculation and values plotted.

When tested on T-cytoplasm corn, the mutant produces race T type symptoms but the disease develops more slowly than with wild type although it produces wild type levels of T-toxin as detected in a microbial assay, suggesting that the
25 reduced virulence is not related to a deficiency in the ability to produce T-toxin. This is clearer on N-cytoplasm corn where the mutant produces lesions significantly smaller than those produced by wild type. When the mutant was crossed to a wild type race O tester, the small lesion phenotype and ability to produce T-toxin segregated independently, indicating that mutant phenotype is
30 not associated with the reduced fitness trait tightly linked with the *ToxI* locus (Klittich et al., Phytopathology, 76:1294 (1986)). The statistical evaluation of

lesion size in the wild type race O genetic background indicates that the mutation causes 60% reduction in the fungal virulence to corn plants. Table 3 depicts the statistical analysis that 86% of the mutant lesions are less than 4 mm in length (average size of 3.5 mm), 60% reduced compared to that of wild type (8.5 mm).

5

Table 3

| <u>Strain</u> | <u>Frequency</u> | | | <u>Lesion size (mm)</u> | | |
|---------------|------------------|-----|------|-------------------------|-----------|----|
| | 1-4 | 5-8 | 9-12 | <u>Mean</u> | <u>SD</u> | |
| WT | 0 | 52 | 48 | 8.5 | 1.0 | A* |
| R45 | 86 | 14 | 0 | 3.5 | 0.9 | B |

*Significant difference at $P < 0.01$.

10 The mutant phenotype is caused by a tagged, single site mutation. In crosses between the mutant and wild type testers, progeny segregated 1:1 for parental types only and all hygromycin B-resistant progeny produced lesions similar to the mutant parent; all hygromycin B-sensitive progeny produced wild type lesions, indicating that a tagged mutation is responsible for the reduced pathogenicity of the mutant. Table 4 depicts the progeny segregation data.

15

Table 4

| Cross | Progeny | path | Parental type | | Nonparental type | |
|----------------|---------------|------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | | PATH hygB ^R | path hygB ^S | PATH hygB ^R | path hygB ^S |
| R.C4.2696 x C5 | random spores | | 24 | 22 | 0 | 0 |
| 1301-R33* x C5 | tetrad1 | | 4 | 4 | 0 | 0 |
| | tetrad2 | | 4 | 4 | 0 | 0 |
| | tetrad3 | | 4 | 4 | 0 | 0 |
| | Random spores | | 21 | 22 | 0 | 0 |

35

*13012-R33 (path, hygB^R, Tox⁻, MAT-2) is a progeny from the first cross, carrying the R.C4.2696 mutation.

Example 2

Cloning, Sequencing and Characterization of DNA Flanking the REMI Vector Insertion Site

5 A total of 11.3 kb of genomic DNA surrounding the insertion site was cloned and completely sequenced (SEQ ID NO:59; Figure 2). The sequence was derived from seven plasmid clones. The first three (p2l4B7, p2l4Ml and p2l4Sl) were recovered from the tagged site in mutant R.C4.2696 and cover about 60% (6.6 kb) of the entire region. The rest (p118B14, p118BC4, p9P2 and p12H6) 10 were recovered from transformants generated using the chromosome walking strategy. DNA to the left of the insertion site (3.4 kb) was cloned on p2l4B7; DNA on the right (7.9 kb) was cloned on different overlapping plasmids. p9P2 carries the largest amount (4.6 kb) including genomic DNA on p12H6.

 Analysis of the combined sequences revealed two open reading frames 15 (ORFs). ORF1 (5.4 kb) starts 576 bp upstream of the REMI vector insertion site and ends with an in-frame stop codon (TAG) 3029 bp from the end of the sequenced region in the right flank. No "TATA" box-like element is found in the expected position, but five putative "CAAT" boxes are located upstream of the start codon (ATG), three of them are in the range found in most filamentous 20 fungal promoters (60-200 bp) (Gurr et al., 1987, *infra*). Sequence around ATG of ORF1 (CACCATGCT) (SEQ ID NO:38) is similar to the fungal consensus (CACCATGGC) (SEQ ID NO:39). Although there are several ATGs found upstream, they are less likely to be used as a start codon because the surrounding sequences lack similarity to the consensus. Three putative introns are identified 25 by their conserved 5' and 3' border sequences and potential branch sites (Table 5). Splicing these introns eliminated stop codons which would otherwise interrupt the 5.4 kb open reading frame. Three introns have similar size (45-53 bp respectively) which is in the range of intron size determined from most fungal genes. A putative polyadenylation signal (ATAA) is found 223 bp downstream 30 of the translation termination site.

The G+C content of ORF1 is 51.5%, which is similar to most

Cochliobolus genes (Turgeon et al., Mol. Gen. Gene., 238:270 (1993); VanWert et al., Curr. Genet., 22:29 (1992); Yang et al., Plant Cell, 8:2139 (1996); Rose et al., 1996, *supra*). Interestingly, ORF1 is flanked by two regions of G+C rich DNA. The first (1.4 kb, 60.3% G+C) is found between ORF1 and ORF2; the
5 second (1.2 kb, 60.3% G+C) is found 1.8 kb downstream of the stop codon of ORF1. Database searches using the translated protein sequence of ORF1 revealed high similarity to SafB, one of the multifunctional enzymes catalyzing the biosynthesis of the cyclic peptide antibiotic saframycin Mx1 produced by the bacterium *Myxococcus xanthus* (Pospiech et al., Microbiology, 142:741 (1996)).

10 The entire nucleotide sequence of ORF1 (CPS1) is designated SEQ ID NO:2 (6,550 base pairs from the 11.3 kb sequenced region, Figure 2). The deduced amino acid sequence of CPS1 protein is designated SEQ ID NO:3. A modification of the ChCPS1 sequence, including changes in three base pairs ("ATG" added between positions 5349 and 5350 of the GenBank entry
15 (GenBank Accession number AF332878)) and an addition of 31 amino acids (the first thirty amino acids ("MMGNYAFNPDNQQSYDGQFGSPGEASRRST")) were added at the N-terminus based on the selection of a new start codon and an additional methionine ("M" at position 1489 was missing in the Genbank entry)) is designated SEQ ID NO:50 (6553 base pairs). The deduced amino acid
20 sequence of the modified ChCPS1 protein is designated SEQ ID NO:185 (1774 amino acids; revised version of the original CPS1 protein (GenBank Accession number AAG53991)). The open reading frame is 5,474 base pairs (736-6209), a 93 base pair increase compared to the deposited sequence that was 5,381 bp. A new start codon (position 736, the original one at position 826) was proposed
25 based on the amino acid alignment of several CPS1 orthologs from different fungi that revealed conserved residues in this region. The stop codon (6,209) is the same as the original GenBank sequence.

Table 5. Characteristics of putative introns in *CPS1* and *TES1*

| Gene | Intron | Size (bp) | Location | 5' Border | 3' Border | Branch Site |
|------|--------|-----------|-----------|-----------------------------------|-------------------|-------------|
| CPS1 | I | 45 | 3060-3105 | GTAAGT | TAG | GTCTAAC |
| | II | 51 | 4532-4582 | GTAAGT | CAG | TGCTAAC |
| | III | 53 | 5187-5239 | GTACGT | CAG | TACTAAC |
| TES1 | I | 49 | 528-566 | GTAAGT | TAG | CCTTAAG |
| Cons | | | | GTA ^A /C ^{GT} | T/C ^{AG} | YNCTAAC* |

ORF2 starts about 1.6 kb upstream of the start codon of *CPS1* and is transcribed in the opposite direction (Figure 2). No "TATA" box-like element and CAAT box are found; instead, an AT-rich sequence "AAACTAT" is located 11 bp upstream of the start codon ATG and a CT motif is found in the -30 region, which is characteristic of a number of fungal genes that lack a CAAT box in their promoter region (Gurr et al., In: Gene Structure in Eukaryotic Microbes, Vol.22, published by the Society for General Microbiology, Oxford, England: IRL Press, Kinghorn, ed., pp 93-140 (1987)). The sequence around ATG matches perfectly fungal gene consensus. A putative intron (50 bp) is found in the middle of ORF2 with conserved 5' and 3' border sequences and a potential branch site (Table 5). A putative polyadenylation signal (AAATA) is found 189 bp downstream of the translation stop codon TGA. The G+C content of ORF2 is 55.5%, which is slightly higher than the normal range because the 5' end of ORF2 is located in the region of G+C rich DNA upstream of ORF1. Database search revealed that ORF2 encodes a protein with high similarity to *Homo sapiens* thioesterase II (hTE, Liu et al., J. Biol. Chem., 272:13779 (1997)) and *E. coli* thioesterase II encoded by the *tesB* gene (Naggert et al., J. Biol. Chem., 266:11044 (1991)). The nucleotide sequence of ORF2 (*TES1*) is designated SEQ ID NO:57. The deduced amino acid sequence of the *TES1* protein is designated SEQ ID NO:58.

Modular structure of CPS1. Predicted CPS1 protein (1743 amino acids, M_r 193235) contains two structurally similar modules, both of which are similar to SafB1, the first module of saframycin synthetase B (overall 25% identity; 50%

similarity) and have apparent amino-acid-activating and thiolation domains but lack methyltransferase activity, thus appearing to be typical type I modules (Figure 3). The number of amino acids in each module is different: the first module (CPS1A) consists of 574 amino acids (from the first residue of core 1 to the last residue of core 6), which is larger than most type I modules; the second module (CPS1B) has 530 amino acids, which is average. The distance between the two modules is 193 amino acids, much shorter than most peptide synthetases (500-600 amino acids), but this distance is not highly conserved, i.e., an opposite variation is found in HC-toxin synthetase and cyclosporine synthetase, both of which have about 1,000 amino acids between the first and second amino-acid-activating module (see Table 6F).

Tables 6A-F show a comparative alignment of core amino acid sequences in CPS1A and CPS1B with those of other peptide synthetases. In each of Tables 6A-F, the first column shows the names of peptide synthetases; the second indicates the position of the first residue aligned in the original amino acid sequence of each protein; the last column on the right indicates the number of amino acids between two cores (6A-E, in parentheses) or the distance between two adjacent amino-acid-activating modules (Table 6F, in parentheses). The extra column in 6F, shows the total number (underlined) of residues in each amino-acid-activating module in which the aligned core sequence is located. The consensus of each core sequence is on the top, which includes identical or similar residues found in all peptide synthetases or with only a few exceptions (active site also indicated by asterisks). SafB1: the first module in saframycin Mx1 synthetase B of *Myxococcus xanthus* (Genbank Accession No. U24657); GrsA: gramicidin S synthetase A of *Bacillus brevis* (SWISS PROT Accession No. P14687); HTS1A and HTS1B: the first two modules in HC-toxin synthetase of *Cochliobolus carbonum* (Q01886); EsynA and EsynB: two modules in enniatin synthetase of *Fusarium scirpi* (EMBL Accession No. Z18755); ACVA and ACVB: the first two modules in ACV synthetase of *Aspergillus nidulans* (SWISS PROT P19787); CysnA and CsynB: the first two modules in

cyclosporine synthetase of *Tolypocladium nivenm* (EMBL Accession No. Z28383).

Table 6A: A Comparative Amino Acid Sequence Alignment of the Amino-Acid-Activating Domain (Core 1).

| Consensus | X L K A G X X X V P I D P X X | | | | | | | | | | | | | | | SEQ ID NO: 73 | | |
|-----------|-------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---------------|------|---------------|
| | 10 | | | | | | | | | | | | | | | | | |
| CPS1A | 165 | C | F | I | A | G | V | V | A | V | P | I | N | S | V | D | (74) | SEQ ID NO: 61 |
| CPS1B | 931 | C | F | V | L | G | A | V | C | I | P | M | A | P | I | D | (74) | SEQ ID NO: 62 |
| SafB1 | 96 | C | L | Y | A | G | V | V | A | V | P | V | Y | P | P | D | (77) | SEQ ID NO: 63 |
| GrsA | 109 | V | L | K | A | G | - | G | Y | V | P | I | D | I | E | Y | (77) | SEQ ID NO: 64 |
| HTS1A | 301 | I | L | K | A | G | G | V | C | V | P | I | D | P | R | Y | (82) | SEQ ID NO: 65 |
| HTS1B | 1906 | V | V | Q | A | G | G | V | F | V | L | L | E | P | G | H | (80) | SEQ ID NO: 66 |
| EsynA | 556 | V | L | K | A | G | H | A | F | T | L | I | D | P | S | D | (63) | SEQ ID NO: 67 |
| EsynB | 1626 | I | L | K | A | N | L | A | Y | L | P | L | D | V | R | S | (65) | SEQ ID NO: 68 |
| ACVA | 361 | V | W | K | S | G | A | A | Y | V | P | I | D | P | T | Y | (76) | SEQ ID NO: 69 |
| ACVB | 1455 | V | W | K | S | G | G | A | Y | V | P | I | D | P | G | Y | (67) | SEQ ID NO: 70 |
| CsynA | 556 | I | L | K | A | H | L | A | Y | L | P | L | D | I | N | V | (70) | SEQ ID NO: 71 |
| CsynB | 1642 | I | L | K | A | G | H | A | Y | L | P | L | D | V | N | V | (68) | SEQ ID NO: 72 |

Table 6B: A Comparative Amino Acid Sequence Alignment of the Amino-Acid-Activating Domain (Core 2).

| Consensus | 10 | | | | | | | | | | | | | | | | | | SEQ ID NO:74 | | | |
|-----------|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|--------------|---|-------|--------------|
| | F | T | S | G | X | T | G | X | P | K | G | V | X | X | H | R | X | I | | | | |
| CPS1A | 253 | F | S | R | A | P | T | G | D | L | R | G | V | V | L | S | H | R | T | I | (312) | SEQ ID NO:75 |
| CPS1B | 1019 | W | T | Y | W | - | T | P | D | Q | R | A | V | Q | L | G | H | S | Q | I | (226) | SEQ ID NO:76 |
| | | | | | | | | | | * | | | | | | | | | | | | |
| SafB1 | 187 | Y | T | S | G | S | T | A | D | P | K | G | V | V | L | T | H | R | N | L | (213) | SEQ ID NO:77 |
| GrsA | 190 | Y | T | S | G | T | T | G | N | P | K | G | T | M | L | E | H | K | G | I | (166) | SEQ ID NO:78 |
| HTS1A | 397 | F | T | S | G | S | T | G | V | P | K | C | I | V | V | T | H | S | Q | I | (154) | SEQ ID NO:79 |
| HTS1B | 2000 | F | T | S | G | - | T | G | V | P | K | G | A | V | A | T | H | Q | A | Y | (166) | SEQ ID NO:80 |
| EsynA | 633 | F | T | S | G | S | T | G | I | P | K | G | I | M | I | E | H | R | S | F | (165) | SEQ ID NO:81 |
| EsynB | 1706 | F | T | S | G | S | T | G | K | P | K | G | V | M | I | E | H | R | A | I | (169) | SEQ ID NO:82 |
| ACVA | 451 | Y | T | S | G | T | T | G | F | P | K | G | I | F | K | Q | H | T | N | V | (172) | SEQ ID NO:83 |
| ACAB | 1538 | Y | T | S | G | T | T | G | R | P | K | G | V | T | V | E | H | H | G | V | (181) | SEQ ID NO:84 |
| CsynA | 640 | F | T | S | G | S | T | G | K | P | K | G | V | M | I | E | H | R | G | I | (172) | SEQ ID NO:85 |
| CsynB | 1724 | F | T | S | G | S | T | G | K | P | K | G | V | M | I | E | H | R | G | V | (174) | SEQ ID NO:86 |

*An insertion (2 residues between R and A) is not shown.

Table 6C: A Comparative Amino Acid Sequence Alignment of the Amino-Acid-Activating Domain (Core 3).

| Consensus | 10 | | | | | | | | | | | | | | | SEQ ID NO:87 | |
|-----------|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|--------------|--------------|
| | G | E | L | X | V | X | G | X | G | L | A | R | G | Y | | | |
| CPS1A | 583 | G | E | I | W | V | D | S | P | S | L | S | G | G | F | (32) | SEQ ID NO:88 |
| CPS1B | 1209 | G | E | I | W | V | Q | S | E | A | N | A | Y | S | F | (25) | SEQ ID NO:89 |
| SafB1 | 418 | G | E | I | W | V | R | G | P | S | V | A | Q | G | Y | (23) | SEQ ID NO:90 |
| GrsA | 374 | G | E | L | C | I | G | G | E | G | L | A | R | G | Y | (23) | SEQ ID NO:91 |
| HTS1A | 569 | G | E | L | L | I | E | S | G | H | L | A | D | K | Y | (31) | SEQ ID NO:92 |
| HTS1B | 2184 | G | E | L | I | I | E | G | S | I | L | C | R | G | Y | (26) | SEQ ID NO:93 |
| EsynA | 816 | G | E | L | V | I | E | S | A | G | I | A | R | D | Y | (30) | SEQ ID NO:94 |
| EsynB | 1893 | G | E | L | V | V | T | G | D | G | V | G | R | G | Y | (32) | SEQ ID NO:95 |
| ACVA | 640 | G | E | L | H | I | G | G | L | G | I | S | K | G | Y | (30) | SEQ ID NO:96 |
| ACVB | 1728 | G | E | L | Y | L | G | G | E | G | V | V | R | G | Y | (30) | SEQ ID NO:97 |
| CsynA | 830 | G | E | L | V | V | S | G | D | G | L | A | R | G | Y | (23) | SEQ ID NO:98 |
| CsynB | 1916 | G | E | L | V | V | T | G | D | G | L | A | R | G | Y | (23) | SEQ ID NO:99 |

Table 6D: A Comparative Amino Acid Sequence Alignment of the Amino-Acid-Activating Domain (Core 4).

| Consensus | Y - R T G D L X R | | | | | | | | | | SEQ ID NO:100 | |
|-----------|-------------------|---|---|---|---|---|---|---|---|---|---------------|---------------|
| | | | | | | | | | | | | |
| CPS1A | 628 | F | L | R | T | G | L | L | G | F | (13) | SEQ ID NO:101 |
| CPS1B | 1301 | Y | V | R | T | G | D | L | G | F | (9) | SEQ ID NO:102 |
| SafB1 | 454 | W | L | R | T | G | D | L | G | F | (11) | SEQ ID NO:103 |
| GrsA | 410 | Y | - | K | T | G | D | Q | A | R | (8) | SEQ ID NO:104 |
| HTS1A | 609 | Y | - | R | T | G | D | L | V | R | (8) | SEQ ID NO:105 |
| HTS1B | 2223 | Y | - | K | T | G | D | L | V | R | (8) | SEQ ID NO:106 |
| EsynA | 860 | Y | - | R | T | G | D | L | A | C | (9) | SEQ ID NO:107 |
| EsynB | 1939 | Y | - | R | T | G | D | R | M | R | (10) | SEQ ID NO:108 |
| ACVA | 684 | Y | - | K | T | G | D | L | A | R | (9) | SEQ ID NO:109 |
| ACVB | 1772 | Y | - | K | T | G | D | L | V | R | (11) | SEQ ID NO:110 |
| CsynA | 866 | Y | - | R | T | G | D | R | A | R | (10) | SEQ ID NO:111 |
| CsynB | 1956 | Y | - | R | T | G | D | R | A | R | (10) | SEQ ID NO:112 |

Table 6E: A Comparative Amino Acid Sequence Alignment of the Amino-Acid-Activating Domain (Core 5).

| Consensus | 10 | | | | | | | | | | 20 | | | | | | | | | | SEQ ID NO:113 | | |
|-----------|------|---|---|---|---|---|---|---|---|---|----|---|---|---|---|---|---|----|---|---|---------------|---------|---------------|
| | L | G | R | X | D | X | Q | V | K | I | R | G | X | R | I | E | L | G | E | V | | E | |
| CPS1A | 645 | L | G | - | - | L | Y | E | D | R | I | R | - | Q | R | V | E | *N | G | Q | L | E (61) | SEQ ID NO:114 |
| GrsA | 427 | L | G | R | I | D | N | Q | V | K | I | R | G | H | R | V | E | L | E | E | V | E (120) | SEQ ID NO:115 |
| HTS1B | 627 | L | G | R | K | D | T | Q | V | K | M | N | G | Q | R | F | E | L | G | E | V | E (162) | SEQ ID NO:116 |
| HTS1A | 2248 | V | G | R | S | D | T | Q | I | K | L | A | G | Q | R | V | E | L | G | D | V | E (163) | SEQ ID NO:117 |
| EsynA | 878 | L | G | R | M | D | S | Q | V | K | I | R | G | Q | R | V | E | L | G | A | V | E (139) | SEQ ID NO:118 |
| EsynB | 1958 | F | G | R | M | D | N | Q | F | K | I | R | G | N | R | I | E | A | G | E | V | E (549) | SEQ ID NO:119 |
| ACVA | 702 | L | G | R | A | D | F | Q | I | K | L | R | G | I | R | I | E | P | G | E | I | E (123) | SEQ ID NO:120 |
| ACVB | 1792 | L | G | R | N | D | F | Q | V | K | I | R | G | L | R | I | E | L | G | E | I | E (116) | SEQ ID NO:121 |
| CsynA | 884 | F | G | R | M | D | Q | Q | V | K | I | R | G | H | R | I | E | P | A | E | V | E (149) | SEQ ID NO:122 |
| CsynB | 1970 | F | G | R | M | D | H | Q | V | K | V | R | G | H | R | I | E | L | A | E | V | E (561) | SEQ ID NO:123 |
| CPS1B | 1397 | L | G | S | I | G | D | T | F | E | V | N | G | L | N | H | F | S | M | D | I | E (96) | SEQ ID NO:124 |
| SafB1 | 1662 | S | G | R | R | K | D | L | L | V | I | R | G | R | N | Y | Y | P | Q | D | L | E (153) | SEQ ID NO:125 |

*An insertion (two amino acid) between E and N in CPS1A is not shown.

The less conserved cores 5 in CPS1B and SafB1 are indicated by arrows.

Table 6F: A Comparative Amino Acid Sequence Alignment of the Thioester Formation Domain (Core 6).

| Consensus | F F X X G G D S L X A X X | | | | | | | | | | | | | | SEQ ID NO:126 | | |
|-----------|---------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---------------|--------|---------------|
| | 10 | | | | | | | | | | | | | | | | |
| CPS1A | 726 | L | D | I | P | F | L | D | S | L | S | E | R | C | <u>574</u> | (193) | SEQ ID NO:127 |
| CPS1B | 1448 | R | D | P | N | G | Q | D | S | Q | M | I | T | E | <u>530</u> | | SEQ ID NO:128 |
| SafB1 | 645 | L | P | D | L | G | L | D | S | L | A | L | V | E | <u>562</u> | (590) | SEQ ID NO:129 |
| GrsA | 567 | F | Y | A | L | G | G | D | S | I | K | A | I | Q | <u>471</u> | | SEQ ID NO:130 |
| HTS1A | 812 | F | I | H | A | G | G | D | S | I | T | A | M | Q | <u>524</u> | (1082) | SEQ ID NO:131 |
| HTS1B | 2422 | F | F | S | S | G | G | N | S | M | A | A | I | A | <u>529</u> | | SEQ ID NO:132 |
| EsynA | 1040 | F | F | E | M | G | G | N | S | I | I | A | I | K | <u>497</u> | (906) | SEQ ID NO:133 |
| EsynB | 2530 | F | F | Q | L | G | G | H | S | L | L | A | T | K | <u>917**</u> | | SEQ ID NO:134 |
| ACVA | 848 | F | F | R | L | G | G | H | S | I | T | C | I | Q | <u>500</u> | (595) | SEQ ID NO:135 |
| ACAB | 1931 | F | F | S | L | G | G | D | S | L | K | S | T | K | <u>489</u> | | SEQ ID NO:136 |
| CsynA | 1053 | F | F | D | L | G | G | H | S | L | T | A | M | K | <u>510</u> | (577) | SEQ ID NO:137 |
| CsynB | 2551 | F | F | N | V | G | G | H | S | L | L | A | T | K | <u>922**</u> | | SEQ ID NO:138 |

*Active site for 4'-phosphopantetheine binding.

**Type II modules containing a methyltransferase domain (about 400 amino acids) between cores 5 and 6. All others are type I modules without this insertion.

Amino acid alignment of the two modules of CPS1 to SafB1 indicated that these modules are highly similar to each other in both overall amino acid composition and conserved motif sequences as defined by Stachelhaus and Marahiel (Stachelhaus et al., 1995, *supra*; Marahiel, 1997, *supra*). When aligned to other bacterial or fungal peptide synthetases, CPS1 only showed local similarity to cyclosporine synthetase (Weber et al., Current Genetics, 26(2):120 (1994)) and tyrocidine synthetase A (Mootz et al., J. Bacteriol., 179(21):6843 (1997)), but when the amino acids in motif regions were aligned, an overall conservation was observed. Both CPS1A and CPS1B have all five core sequences in the amino-acid-activating domain (Table 6A-E). Cores 3 and 4 are well conserved except for the replacement of an aspartic acid residue of core 4 by a leucine in CPS1A. Cores 1, 2 and 5 show weak conservation, but similar variations are also seen in SafB1. A thiolation domain is found in both modules, which contains a highly conserved motif (core 6, Table 6F). The serine residue in this motif has been shown to be the active site for 4'-phosphopantetheine attachment (Schlumbohm et al., J. Biol. Chem., 266:23135 (1991); Stein et al., FEBS Lett., 340:39 (1994)).

The distances between the six core sequences in the two modules are also largely conserved. Two exceptions are found in the first module, which has 312 amino acids between cores 2 and 3, larger than normal (150-200); 61 between cores 5 and 6, only half of that of most peptide synthetases. SafB1 also shows distance variations at these two interval regions (Table 6B and E). In addition to amino-acid-activating and thiolation domains, CPS1 also has an integrated thioesterase domain (TE) in the carboxy-terminal end of CPS1B (Figure 12). A signature sequence GX SXG (SEQ ID NO:147), which is highly conserved in animal fatty acid thioesterase type II enzymes and several peptide synthetases, is found in this domain (Table 7).

Table 7: Comparative Alignment of Amino Acid Sequences of Active Sites of Thioesterase Domains (TE) in CPS1 with those of other Peptide Synthetases.

| Consensus | X | X | X | G | X | S | X | G | X | S | X | X | X | A | F | E | X | SEQ ID NO:139 | |
|-----------|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---------------|---------------|
| | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | | |
| | 10 | | | | | | | | | | | | | | | | | | |
| CPSI-TE | 1619 | V | L | R | P | G | P | S | S | G | S | E | Q | H | D | Q | A | (125) | SEQ ID NO:140 |
| ACVA-TE | 3621 | Y | H | F | I | G | W | S | F | G | G | T | I | A | M | E | I | (168) | SEQ ID NO:141 |
| GrsB-TE | 4267 | Y | V | L | I | G | Y | S | S | G | G | N | L | A | F | E | V | (186) | SEQ ID NO:142 |
| GrsT-TE | 1117 | F | A | F | L | G | H | S | M | G | A | L | I | S | F | E | L | (157) | SEQ ID NO:143 |
| SafA-TE | 6313 | L | T | L | F | G | Y | S | A | G | C | S | L | A | F | E | A | (173) | SEQ ID NO:144 |
| TycC-TE | 93 | Y | T | L | M | G | Y | S | S | G | G | N | L | A | F | E | V | (163) | SEQ ID NO:145 |
| TycF-TE | 76 | F | A | F | F | G | H | S | M | G | G | L | V | A | F | E | L | (168) | SEQ ID NO:146 |

ACV: ACV synthetase (SWISS PROT Accession No. P19787); GrsB: gramicidin S synthetase B (P14688); GrsT: the thioesterase encoded by grsT (P14686) in gramicidin S synthetase gene cluster; SrfA: surfactin synthetase A-3 (Q08787); TycC: tyrocidine synthetase C (Genbank Accession No. AF004835); TycF: the thioesterase encoded by tycF (AF004835) in the tyrocidine synthetase gene cluster. The highly conserved residues (GX SXG; SEQ ID NO:147) are indicated by asterisks. The number on the left of each amino acid sequence indicates the original position of the first residue; the number on the right (in parentheses) indicates the distance between the last residue shown to the end of each protein.

Sequence homology analysis of TES1 protein. The predicted TES1 protein consists of 367 amino acids (M_r 41013) amino acid alignment of TES1 to hTE, TESB and *Mycobacterium tuberculosis* TESB homolog (Philipp et al., Proc. Natl. Acad. Sci. USA, 93:3132 (1996)) showed that these proteins have an overall 40% identity and 60% similarity. A highly conserved VHS motif (putative active site) is found in the C-terminal region of TES1 at a conserved position (Figure 13). All these thioesterases have no sequence similarity with the previously identified animal type I or type II thioesterases known to be involved in the chain termination of fatty acid synthesis (Naggert et al., J. Biol. Chem., 266:11044 (1991)). Interestingly, TES1 has more homology to hTE than to two bacterial genes, suggesting that both proteins belong to a new family of eukaryotic thioesterases.

Targeted disruption of CPS1. Disruption of either CPS1A or CPS1B restored the original mutant phenotype. Ten transformants from each of four individual disruption experiments using different constructs, including the plasmid recovered from the REMI insertion site in the mutant (p214B7) and three vectors for chromosome walking (p214SNP, p118BSP and p118BCS) were purified and assayed on N-cytoplasm corn. All transformants showed the same small lesion phenotype as that of the original REMI mutant. Southern blot analysis confirmed that all transformants showing the mutant phenotype resulted from homologous integration of the transforming vector that disrupted the wild type *CPS1*. No transformants showing the wild type phenotype were obtained, presumably because of the large genomic DNA fragments (over 800 bp in all disruption experiments) on the transforming vector that resulted from high efficiency of homologous recombination and the low chance to recover transformants with ectopic integration.

Example 3

Targeted disruption of CPS1 homolog in *C. victoriae*

Methods and Materials

- Strains, growth conditions and transformation. Strains of *Cochliobolus* species and relatives used for genomic DNA hybridization are listed in Table 8. The strain HyW, a victorin-producing isolate of *C. victoriae* was recovered from storage and grown on CMX medium (Turgeon et al., Mol. Gen. Genet., 201:450 (1985)) for conidiation or on oat meal agar medium (Churchill et al., Fungal Genet. Newsl., 42A:41 (1995)) for victorin detection at 24°C under warm white lights (Sylvania Inc., Danvers, MA). Transformation was done using the *C. heterostrophus* procedure (Turgeon et al., Mol. Gen. Gene., 238:270 (1993)).

Table 8. Detection of CPS1 homologs in *Cochliobolus* spp and relatives

| Strain ^a | Host ^b | <i>Eco</i> RI digest ^c | Hybridization <i>Hind</i> III digest ^d | <i>Bgl</i> III digest ^e |
|------------------------------------|--------------------------------|-----------------------------------|--|------------------------------------|
| <i>C. heterostrophus</i> | Corn | | | |
| race T (C4) | (Turf-13) | + | 5.2 3.2 | 4.2 |
| race O (C5) | | + | 5.2 3.2 | 4.2 |
| | | | | |
| <i>C. carbonum</i> | Corn ¹ | | | |
| race 1 (26R13) | (hm1hm1) | + | 6.6 | 5.0 |
| race 2 (Yug Y) | | N | 6.6 | 5.0 |
| race 3 (BZ1703)* | | N | 6.6 | 5.0 |
| | | | | |
| <i>C. victoriae</i> (HvW) | Oats (Vb) | + | N | 5.0 |
| <i>C. sativus</i> (A20) | Grasses ² | + | 3.0 | N |
| <i>C. specifer</i> (D5-7) | Grasses ² | + | N | N |
| <i>C. homomorphus</i> (ATCC 13409) | Unknown | N | 5.8 | N |
| <i>C. dactyloctenii</i> (7938-9) | Unknown | N | 5.9 | N |
| <i>S. turcica</i> (NK2) | Sorghum and maize ³ | + | N | N |
| <i>S. rostrata</i> (32197) | Weeds and bamboo ⁴ | + | 2.8 | N |
| <i>B. sacchari</i> | Sugarcane ⁵ | | | |

| | | | | |
|-----------|--|---|---------|---|
| (764-1) | | + | 5.4 2.5 | N |
| (1249-10) | | N | 5.4 2.5 | N |

- a. C.= *Cochliobolus*. S.= *Setosphaeria*. B.= *Bioplaris*. The name of isolates (or lab strains) of each species are given in parentheses and those known to produce host-specific toxins are underlined. *Provided by Tsukiboshi Takao (Japan) and the isolate could be either BZ1209 or BZ1703.
- b. Genotype susceptible to the host-specific toxin-producing isolate is given in parentheses. References for hosts of those species not mentioned are as follows: 1: Welz et al., Phytopathology, 83:593 (1993); Leonard et al., Phytopathology, 80:1154 (1990) (for races 2 and 3 only). 2: Domsch et al., "Compendium of Soil Fungi, Vol. 1," New York, New York:Academic Press, pp 216-222 (1980). 3: David et al., "Fungi on Plants and Plant Products," St. Paul, Minnesota:APS Press, p. 635 (1989); Thakur et al., Plant Dis., 73:151 (1989). 4: Rao et al., Indian Bot. Rep., 6:38 (1987); Bhat et al., Curr. SCI. (BANGALORE), 58:1148 (1989). 5: Yoder, Ann. Rev. Phytopathol., 18:103 (1980).
- c. Genomic DNAs (from a previously prepared gel blot filter, Rose et al., 1996, *supra*) were probed with the 3.4 kb *CPSI* fragment cloned on p214B7. "+" indicates a strong hybridization signal. All species hybridized to a large fragment (about 23 kb).
- d. Genomic DNAs selected from a collection were probed with the *CPSI* 3.2 kb fragment cloned on p214S1. The size of fragments that hybridized to the probe is given in kb. The intensities of hybridization signals were similar to each other. N = not done.
- e. Genomic DNAs were probed with the same *CPSI* fragment as in c.
- DNA manipulations and targeted disruption of the CPS1 homolog of *C. victoriae*. Genomic DNAs for probing were prepared according to Yoder, In: Genetics of Plant Pathogenic Fungi, Vol. 6, San Diego, California:Academic Press, Sidhu, ed., pp. 93-112 (1988)), or selected from a lab DNA collection (stored at 4°C). A gel blot filter bearing known genomic DNAs was also probed. Plasmid DNA preparation, restriction enzyme digestions, gel electrophoresis, gel blot analysis were done using standard protocols (Sambrook et al., 1989, *supra*). For probing, *CPSI* fragments of *C. heterostrophus* cloned on p214B7 (3.4 kb left flank) and p214S1 (3.2 kb right flank) were prepared by restriction enzyme digestion of the plasmid DNAs followed by purification using the QIAquick Gel Extraction Kit (QIAGEN Inc., Chatsworth, CA). The plasmid p18B14, which carries the 2.3 kb *Bgl*II fragment of *CPSI* interrupted by the *hygB* cassette was linearized with *Bgl*II and introduced into HvW genome.

Transformants were purified by isolation of single conidia and genomic DNAs were digested with *Bgl*II and probed with the *CPSI* 3.2 kb fragment.

Bioassays. Pathogenicity was determined by an oat plant assay. Fungal strains were grown in individual oat meal agar medium plates (60 X 15 mm) containing hygromycin B (60 µg/ml) for 10 days at 24°C under lights. Conidia were scraped from the plates and suspended in 6 ml sterilized distilled water. One ml of conidial suspension of each strain was mixed with 60 seeds of susceptible or resistant oats. Inoculated seeds were planted in 4" X 6" pots and seedlings were allowed to grow for two weeks. Seed germination rate and symptom development were recorded at different stages (4, 6, 8 and 24 days after inoculation). Detection of victorin production using HPLC analysis was done by Alice Churchill in Dr. Vladimir Macko's lab at Boyce Thompson Institute for Plant Research.

Results

Detection of *CPSI* homologs. Genomic DNAs of 12 isolates (or lab strains) of 9 fungal species hybridized to *CPSI* (Table 8). All 6 *Cochliobolus* species, including 4 known plant pathogens (*C. carbonum*, *C. victoriae*, *C. sativus* and *C. specifer*) and 2 species with unknown hosts (*C. homomorphus* and *C. dactyloctenii*) gave hybridization signals of the same intensity as that of *C. heterostrophus CPSI* fragments. Two phytopathogenic *Setosphaeria* species and *Bioplaris sacchari*, a sugarcane pathogen gave a similar hybridization intensity.

CPSI homologs appear to be polymorphic among different species, i.e., all species gave one or two unique bands when *Bgl*III or *Hind*III digested genomic DNAs were probed (except for *C. victoriae*, which showed the same hybridization pattern as *C. carbonum*) (Table 8). Interestingly, *Eco*RI digested genomic DNAs of the same species did not show polymorphisms; all species hybridized to a large fragment (about 23 kb, Table 8), indicating the absence of an *Eco*RI site in all *CPSI* homologs as in the *C. heterostrophus* gene. In *C. heterostrophus*, a >12 kb of genomic region which includes *CPSI* (5.4 kb), *TES1* (1.1 kb) and sequence downstream of the 3' end of *CPSI* has no *Eco*RI sites. In contrast to species-dependent polymorphisms, *CPSI* homologs appear to

be highly conserved among different isolates of the same species. Both *C. heterostrophus* race T and race O hybridized to the same 4.2 kb *Bgl*II fragment (or 5.2 and 3.2 kb *Hind*III fragments); all three *C. carbonum* races hybridized to the same 5.0 kb *Bgl*II fragment (or 6.6 kb *Hind*III fragment) (Table 8) and *B. sacchari* isolates 764-1 and 1249-10 hybridized to the same *Hind*III fragments (5.4 and 2.5 kb) (Table 8).

Twenty transformants were obtained from transformation of the victorin-producing isolate HvW with *Bgl*II-linearized plasmid p118B14. Six transformants were purified and assayed for both victorin production and pathogenicity to susceptible oat plants. All transformants produced wild type levels of victorin as determined by HPLC analysis, but four of them (Tx7, Tx2, Tx5 and Tx8) showed dramatically reduced virulence in the plant assay. The seed germination rate on the eighth day after inoculation is only 13-25% for wild type and two transformants (Tx9 and Tx4), but 45-63% for the other four transformants. One day 24 after inoculation, all plants emerged from the seeds inoculated with wild type, Tx9 or Tx4 were killed but most (29-63%) from the seeds inoculated with Tx2, Tx7, Tx5 or Tx8 still survived (Table 9). Southern blot analysis confirmed that transformants showing the reduced virulence phenotype resulted from homologous integration of the transforming vector that disrupted the wild type *CPSI* homolog in *C. victoriae* genome; transformants showing the wild type phenotype resulted from ectopic integration events that left the native gene intact. All transformants remained nonpathogenic to resistant oats, indicating that disruption of the *CPSI* homolog does not affect host specificity of the fungus.

Table 9. Disease development of oat plants inoculated with *C. victoriae* transformants (Tx).

| Strain ^a | No. germinated ^b | | | Germination Rate (%) ^c | No. survivors ^d | |
|---------------------|-----------------------------|----|----|-----------------------------------|----------------------------|-----|
| | 4 | 6 | 8 | | 24 | % |
| Control-1 | 28 | 41 | 45 | 75 | 75 | 100 |
| Control-2 | 40 | 50 | 50 | 83 | 50 | 100 |
| Control-3 | 1 | 7 | 12 | 20 | 0 | 0 |
| Tx2 | 8 | 26 | 27 | 45 | 16 | 59 |

| Strain ^a | No. germinated ^b | | | Germination Rate (%) ^c | No. survivors ^d | |
|---------------------|-----------------------------|----|----|--------------------------------------|----------------------------|----|
| | 4 | 6 | 8 | | 24 | % |
| Tx4 | 5 | 15 | 15 | 25 | 0 | 0 |
| Tx5 | 2 | 24 | 28 | 47 | 8 | 29 |
| Tx7 | 14 | 36 | 38 | 63 | 24 | 63 |
| Tx8 | 7 | 29 | 29 | 47 | 13 | 47 |
| Tx9 | 0 | 3 | 8 | 13 | 0 | 0 |

- a. Control-1 = uninoculated susceptible oat seeds. Control-2 and Control-3 = resistant and susceptible oat seeds inoculated with wild type *C. victoriae* (isolate HvW), respectively. Six transformants were tested on both resistant and susceptible seeds, but only data for the later are shown (all transformants gave the same results as Control-2 when tested on resistant seeds). Repeat experiments gave similar results (data not shown).
- b. Sixty oat seeds were used for each strain. Emerged oat plants were counted 4, 6 and 8 days after inoculation.
- c. Calculation based on the data collected on the day 8.
- d. Recorded on day 24 after inoculation. The percentage of survivors is based on the number of plants recorded on days 8 and 24.

15 Discussion

CPS1 encodes an enzyme with an adenylation domain. A gene designated *CPS1* was cloned from the corn pathogen *C. heterostrophus* using the REMI mutagenesis procedure. Structural and functional analyses strongly suggest that *CPS1* encodes an enzyme with one or more adenylation domains, e.g., a CoA ligase. CPS1 contains two repeated functional units with a modular organization, and has a thioesterase motif (GX SXG; SEQ ID NO:147). This motif has been demonstrated to be an active site for catalyzing release of medium-chain-length (C₈₋₁₂) fatty acids in fatty acid synthases and potentially for termination of peptide chains or for repeated acyl transfer reactions because the same motif is also the characteristic of acyl transferases or acyl transfer domains (AT) of fatty acid synthases (FAS) and polyketide synthases (PKS) (Krättschmar et al., *J. Bacteriol.*, 171, 5422, (1989)).

Although similar TE domains are found in certain fungal PKSs, i.e., *Aspergillus nidulans* *pksL1* gene (Feng and Leonard, *J. Bacteriol.*, 177, 6246, (1995)) and *pksST* gene (Yu and Leonard, *J. Bacteriol.*, 117, 4792, (1995)),

CPS1 is unlikely to be a polyketide synthase because: 1) it does not show any significant similarity to known PKSs, and 2) it lacks unique functional domains found in these proteins such as the ketoacyl synthase domain (KS) and the acyl transferase domains (AT) found in the *N*-terminal region of all fungal PKSs
 5 (Yang et al., 1996, *supra*). This does not exclude the possible common evolutionary origin of CPS1 and PKSs (Stachehaus and Marahiel, 1995, *supra*).

CPS1 could be responsible for biosynthesis of an unidentified peptide phytotoxin. It is well known that several *Cochliobolus* species and related filamentous fungi produce peptide toxins. These include *C. carbonum* and *C.*
 10 *victoriae*, two species most closely related to *C. heterostrophus*. The former produces HC-toxin as mentioned above; the latter produces victorin, a chlorinated cyclized peptide. *Alternaria alternata*, a plant pathogenic species from a genus closely related to *Cochliobolus*, is also known to produce several peptide toxins such as AM-toxin, a cyclic tetradepsipeptide produced by *A.*
 15 *alternata* apple pathotype and tentoxin, a cyclic tetrapeptide produced by *A. alternata* pv. *tenuis* (Nishimura and Kohmoto, 1983). These findings have lead to the postulation that, in addition to T-toxin, *C. heterostrophus* might also produce a similar secondary metabolite, such as a hypothetical "race O" toxin (Yoder, 1981).

20 Interestingly, a *Tox*⁺, *cpsI*⁻ mutant showed reduced virulence on T-cytoplasm corn although it produced the same amount of T-toxin as wild type race T. This is unusual because the interaction between T-toxin and the T-corn-unique URF13 protein is highly specific; the same outcomes should be expected if two strains that produce the same amount of T-toxin attack the same host, T-
 25 corn. The most likely explanation for this result is that the fungal growth *in planta* has been inhibited by the host plant and the poor growth results in reduced T-toxin production which is normal when the fungus is grown in culture. Reduced virulence on T-cytoplasm corn is due to the reduced T-toxin production as that seen in leaky *Tox*⁻ mutants. This inhibition of growth could be
 30 due to the failure of suppression of the host defense mechanism by the fungus, which is mediated by the *CPS1* controlled peptide toxin. A *cpsI*⁻ mutant that

fails to produce this “suppressor” could not be able to colonize plant tissues as vigorously as wild type does, resulting in the reduced ability to cause disease as indicated by the smaller lesion phenotype. If this turns out to be the case, *CPSI* should be considered as a general virulence factor as proposed for ennatin.

5 It is possible that *cpsI*⁻ mutants are still be able to produce a certain amount of CPS1 toxin. One probability is the gene has not been completely inactivated by insertional mutagenesis or targeted disruption. The original REMI insertion occurred at core sequence 1 of CPS1A, a region that might be not critical (function of core 1 is unknown). The second targeted site is located
10 between cores 1 and 2 of CPS1B and the third is located between cores 2 and 3 of the same module. All three insertions do not disrupt critical motifs. On the other hand, *CPSI* contains a number of in-frame start codons and some of them are located immediately downstream of these insertion sites. It is possible that each of these disruptions actually resulted in two subtranscripts, one is
15 transcribed normally from the start codon of *CPSI* and stops at the insertion site and second is transcribed near one of these in-frame ATGs downstream of the insertion site and stops at the end of *CPSI*. Both transcripts could give a truncated protein that still has enzymatic activities. But these separate enzymes might have affinities for their substrates lower than that of holoenzyme. The
20 reduced production of CPS1 toxin might be due to the CPS1 holoenzyme having been split into two fractions by the vector insertion and the resulting truncated proteins being much less active than the original polypeptide. This hypothesis can be tested by construction a *C. heterostrophus* strain in which the entire *CPSI* encoding sequence has been deleted.

25 The second possibility is the existence of multiple copies of *CPSI* in the genome. Previous studies have demonstrated that the gene encoding HC-toxin synthetase (*HTSI*) is duplicated in the genome and both copies (*HTSI-1* and *HTSI-2*) are 270 kb apart in most Tox2⁺ isolates of *C. carbonum* (Ahn and Walton, 1996, *supra*). Disruption of either copy reduced HTS1 activity but did
30 not affect HC-toxin production; when both copies were disrupted, HC-toxin production was abolished (Panaccione et al, 1992, *supra*). But in contrast to the

case of *HTSI*, gel blot analysis does not indicate the presence of a second copy of *CPSI* and disruption of *CPSI* does affect the production of the putative toxin.

It is unlikely that two genes with similar organization are in the genome. An alternative postulation is that there may be a second gene which encodes a protein with the same enzyme activity as CPS1 but does not have significant sequence homology to *CPSI*. This hypothesis is hard to test unless this gene is clustered with *CPSI* and can be recovered by chromosome walking.

In conclusion, pathogenesis by *C. heterostrophus* to corn involves at least two secondary metabolites: the T-toxin, a host specific factor which determines high virulence on a particular host, T-corn and the hypothetical CPS1 toxin, a general factor (either virulence or pathogenicity factor) which contributes to basic mechanisms underlying the disease establishment by the fungus in common host plants.

Example 4

CPSI Orthologs

As described above, *Cochliobolus heterostrophus* gene *CPSI* encodes a putative peptide synthetase that appears to be a general factor for fungal virulence to its hosts. *CPSI* has been found to be highly conserved among at least 9 fungal species belonging to 3 genera including the genus *Cochliobolus* and closely related genera *Bioplaris* and *Setosphaeria*; it has been demonstrated to be required for pathogenesis by three different plant pathogens, i.e., *C. heterostrophus* race O, race T to corn and *C. victoriae* to oats (Lu, 1998, Ph.D. thesis, Cornell University).

To further explore the role of *CPSI* in fungal pathogenesis and its conservation in other fungi, genomic DNAs of additional species of *Cochliobolus* and other closely or distantly related genera were probed with *ChCPSI* by DNA-DNA hybridization (Lu, S.-W., B.G. Turgeon and O.C. Yoder. 1999. Fungal Genetics Conference, March 1999, Pacific Grove, California). Genomic DNAs of 40 field isolates (or lab strains) representing 34 fungal species belonging to 16 genera hybridized when probed with *ChCPSI* (Figure 4).

All 16 *Cochliobolus* species, including the known plant pathogens *C. carbonum*, *C. victoriae*, *C. miyabeanus*, *C. sativus* and *C. specifer*, and five genera closely related to *Cochliobolus*, i.e., *Pyrenophora*, *Setosphaeria*, *Bipolaris*, *Stemphylium* and *Alternaria* showed hybridization intensities comparable to that of *C.*

- 5 *heterostrophus* itself (Figure 4A). DNAs of species from nine distinctly related genera, including several of economic importance (e.g., *Magnaporthe grisea*, *Fusarium graminearum*, *Gaeumannomyces graminis*) or of medical importance (e.g., *Candida albicans*) hybridized weakly to *CPS1* (Figures 4B and 4C) whereas no signal was detected in DNA of the basidiomycete *Ustilago maydis*.
- 10 Homologs of *CPS1* were further identified by polymerase chain reaction (PCR) using degenerate primers designed to conserved regions of *C. heterostrophus CPS1* (*ChCPS1*). Four *CPS1* homologs were cloned and characterized. Three of them were cloned from phytopathogenic fungi, including the wheat head scab fungus *Fusarium graminearum* (*FgCPS1*, 6003 bp, SEQ ID
- 15 NO:40), the potato early blight fungus *Alternaria solani*, (*AsCPS1*, 2369 bp, SEQ ID NO:42) and the barley net blotch fungus *Pyrenophora teres* (*PtCPS1*, 2320 bp, SEQ ID NO:44). The fourth was cloned from the human pathogenic fungus *Coccidioides immitis* (*CiCPS1*, 2435 bp SEQ ID NO:46). The complete *FgCPS1* gene was cloned using both PCR amplification and plasmid rescue
- 20 procedures preceded by targeted gene disruption of this gene in the genome. The remaining three *CPS1* homologs were partially cloned by direct PCR amplification.

- 25 The *FgCPS1* open reading frame (5125 bp) has 50% nucleotide identity to *ChCPS1* in about 4.4 kbp of overlap. No "TATA" box-like element was found in the 5' untranslated region, but other promoter sequences including two putative "CAAT" boxes and a "CT" motif were located upstream of the start codon (ATG). There is only one putative intron found 1508 bp upstream of the stop codon (TGA) in contrast to three in *ChCPS1*.

- 30 A putative polyadenylation signal "AATAA" is located 62 bp downstream of the stop codon. The predicted *FgCPS1* protein (1692 amino acids, M_r 187983 Da, SEQ ID NO:41) has 68% identity, 73% similarity to

ChCPS1 in about a 1,500 amino acid overlap that contains two structurally similar modules highly similar to those of ChCPS1 (Figure 7B). FgCPS1 has no significant similarity to ChCPS1 at the C-terminus, which is shorter and lacks the thioesterase domain seen in ChCPS1.

5 *AsCPS1* (2369 bp, SEQ ID NO:42) has 76% nucleotide identity to *ChCPS1* in the entire cloned region which contains two conserved introns. The translated AsCPS1 protein (partial) includes 758 amino acids (SEQ ID NO:43) corresponding to amino acids 511-1269 in ChCPS1 and has up to 93% identity, 95% similarity to ChCPS1 (Figure 7B).

10 *PtCPS1* (2320 bp, SEQ ID NO:44) has 78% nucleotide identity to *ChCPS1* in the entire cloned region which contains only one intron. The translated PtCPS1 protein (partial) includes 758 amino acids (SEQ ID NO:45) corresponding to amino acids 511-1269 in ChCPS1 and has 93% identity, 96% similarity to ChCPS1.

15 *CiCPS1* (2435 bp, SEQ ID NO:46) has 65% nucleotide identity to *ChCPS1* in the entire cloned region which has no introns. The translated CiCPS1 protein (partial) includes 812 amino acids (SEQ ID NO:47) corresponding to amino acids 511-1040 in ChCPS1 and has 67% identity, 80% similarity to ChCPS1 (Figure 7B). Another ortholog in *Candida* was identified
20 by Southern blot (see Figure 4).

BLAST searches using SEQ ID NO:41 (Figure 6) and SEQ ID NO:47 (Figure 7A) identified orthologs of those fungal CPS1s.

Disruption of *FsCPS1* in *F. graminearum* (= *Gibberella zeae*), the wheat head scab fungus, caused significantly reduced virulence to wheat. All *cps I*⁻ disruptants of *F. graminearum* showed at least 50% (when inoculated with 10⁵/ml conidia) or even 80-90% (when inoculated with 10⁴/ml conidia) reduction in ability to cause a typical "white head" symptom on the host whereas in the same conditions, ectopic transformants caused disease symptoms indistinguishable from wild type. These results suggest that *CPS1* is also
25
30 required for pathogenesis by fungi that are distantly related to *C. heterostrophus*,

arguing that these peptide synthetase gene homologs might control biosynthesis of a general fungal virulence factor.

Discussion

Conservation of CPS1 and taxonomy. By genomic DNA hybridization, 5 *C. heterostrophus* CPS1 homologs were found in 16 additional fungal species belonging to 5 genera. Hybridization signals for some were as strong as the *C. heterostrophus* gene, indicating that CPS1 is highly conserved among these fungi. This conservation appears to match the taxonomic relationships between these species. *Cochliobolus* (anamorph *Bipolaris*) and *Setosphaeria* (anamorph 10 *Exserohilum*) are closely related genera.

Two species, *C. victoriae* and *C. carbonum*, which are able to cross to each other and thus may not be different species (Scheffer et al., 1967; Yoder et al., 1989), showed the same hybridization pattern to CPS1. *B. sacchari*, the closest asexual relative of *C. heterostrophus*, hybridized to two HindIII 15 fragments that were only seen in *C. heterostrophus* itself, but all other species gave only one distinct polymorphic band. Phylogenetic analyses using the internal transcribed spacer (ITS) sequences and fragments of the *GPD* (vanWert and Yoder, 1992) and *MAT* genes (Turgeon et al., 1993, *supra*) also put *C. victoriae/C. carbonum* and *C. heterostrophus/B. sacchari* closest to each other 20 (Turgeon and Berbee, 1997). These results might imply that CPS1 has co-evolved with these genes.

CPS1 homologs and pathogenesis. The genera *Cochliobolus* and *Setosphaeria* include many plant pathogenic species that are commonly associated with leaf spots or blights, mainly on cultivated cereals and wild 25 grasses (Sivanesan, 1987; Alcorn, 1988). This group of phytopathogenic fungi includes both mild pathogens and severe pathogens that often produce host-specific toxins (Yoder, 1980, *supra*). One of the essential questions is whether or not the various diseases on diverse host plants caused by these fungi involve common factors or depend only on individual specific factors, such as host- 30 specific toxins.

Previous studies have shown that host-specific toxins can be critical factors for determining either virulence or host-range, but they do not account for general pathogenicity since they are produced only by certain isolates in the species and the corresponding biosynthetic genes are found only in these toxin-producing isolates (Yoder et al., 1997, *supra*). In contrast, *CPSI* homologs are found in all *Cochliobolus* and *Setosphaeria* species tested so far, suggesting they are a common factor shared by this group. Disruption of the *CPSI* homolog in the oat pathogen *C. victoriae* caused dramatically reduced virulence to victorin-susceptible oats although the transformants produced wild type levels of victorin. This result is similar to that with *C. heterostrophus* race T, in which *cpsI* disruptants still produced wild type levels of T-toxin but showed reduced virulence on T-cytoplasm corn. These results argue strongly that host-specific toxins alone are not sufficient in determining the ultimate outcome of fungus/plant interactions and suggest that the establishment of disease by these fungi also requires CPS1, which might control a pathway for general pathogenicity.

The CPS1 gene cluster and homologs could be fungal “pathogenicity islands”. In the early 1990s, studies on pathogenesis by uropathogenic *E. coli* led to the identification of pathogenicity gene clusters, termed “pathogenicity islands” (Hecker et al., 1990; Blum et al., 1994). Subsequently, similar gene clusters were identified in additional animal or human bacterial pathogens, including *Yersinia pestis*, *Helicobacter pylori* and *Salmonella typhimurium*. These islands often contain genes for production of toxins or genes encoding proteins that are capable of interacting with host defense factors or required for type III secretion systems that deliver virulence proteins into host cells. Usually, they are found only in pathogenic strains (or species); in rare cases, they occur in nonpathogenic strains of the same species or related species (Hacker et al., 1997, *supra*).

In phytopathogenic bacteria, *hrp* gene clusters have been referred to as “pathogenicity islands” because they have several features in common with “pathogenicity islands” in animal pathogenic bacteria, i.e., they are found only in

pathogenic species (required for plant pathogenicity) and contain highly conserved genes (*hrc* genes) defining the type III protein secretion system (Alfano and Collmer, 1996; Barinaga, 1996).

In plant pathogenic fungi, genes or gene clusters with characteristics of “pathogenicity islands” have been identified from certain species, i.e., in *Nectria haematococca*, the *PDA* genes for detoxifying the pea phytoalexin and other pea pathogenicity genes (*PEP*) are located on dispensable chromosomes that are found in all isolates pathogenic to pea but usually absent in all nonpathogenic isolates (VanEtten et al., 1994; Liu et al., 1997, *supra*). In the genus *Cochliobolus*, the *Tox2* gene cluster controlling the biosynthesis of HC-toxin is found only in *C. carbonum* race 1 (pathogenic to *hm1hm1* corn) and the *Tox1* genes controlling T-toxin production are found only in *C. heterostrophus* race T (highly virulent on T-cytoplasm corn); all other races of the same species and all other fungal species tested so far lack these *Tox* genes (Ahn and Walton, 1996, *supra*; Yang et al., 1996, *supra*; Yoder et al., 1997, *supra*).

CPS1 differs in two important ways compared to these fungal “pathogenicity islands”. First, it is highly conserved among several phytopathogenic *Cochliobolus* species and relatives. Second, like certain bacterial “pathogenicity islands”, *CPS1* also has homologs in “nonpathogenic” species. *C. homomorphus* and *C. dactyloctenii*, neither of which causes disease on plants, hybridized strongly to *CPS1*. This may reflect genetic changes in the “pathogenicity island” that resulted in loss of pathogenicity. In the bacterial genus *Listeria*, which includes several human or animal pathogenic species harboring highly conserved “pathogenicity islands”, the “pathogenicity island” homolog in the nonpathogenic species (*L. seeligeri*) was found to be 'silent' due to a mutation that occurred in the promoter region of a critical regulatory gene in the cluster (Hacker et al., 1997, *supra*). These features suggest that the *CPS1* gene cluster and homologs could define a new group of fungal “pathogenicity islands”.

The origin of CPS1. It is known that the evolution of pathogenicity involves two major processes. A pathogenic microorganism could originate

from nonpathogenic progenitors by slow modifications (such as point mutations and genetic recombination) of genes that were adapted for parasitic growth on hosts or by the integration of large fragments of “alien” DNA into the genome that enable the recipient to attack particular hosts (gene horizontal transfer). The latter can occur in the recent or distant evolutionary past. Subsequent vertical transmission in the lineage (if the transferred gene is stable in the recipient genome) would result in the preserve of the gene in all species that diverged after the acquisition of the gene(s) (Scheffer, 1991; Arber, 1993; Krishnapillai, 1996; Burdon and Silk, 1997).

In the past few years, substantial evidence has become available that supports the hypothesis of gene horizontal transfer. All “pathogenicity islands” in animal pathogenic bacteria are believed to have been acquired by a horizontal transfer event (recent or past) because they usually differ in G+C content from the recipient genome and have transposable elements at the boundaries of the gene clusters (Hacker et al., 1997, *supra*). The *hrp* “pathogenicity islands” do not show a significant difference in G+C content or association with transposable elements, but they are also believed to have arisen similarly because *hrc* genes in these “pathogenicity islands” show high similarity to genes defining the type III protein secretion system found in animal pathogenic bacteria as mentioned above (Alfano and Collmer, 1996; and Barinaga, 1996).

Although *CPSI* itself has several typical fungal introns and a G+C content (51.5%) similar to most known fungal genes, genomic regions (about 1.5 kb) flanking the gene have higher G+C content (>60%). Several short G+C-rich regions are also found in the gene cluster; one of the open reading frames (ORF10) has a 63.6 % G+C content. Compared to those filamentous fungal genomes characterized so far, including *N. crassa*, *A. nidulans*, *U. maydis* (all have G+C content 51-54%, see Karlin and Mrázek, 1997, *supra*), the genomic region around *CPSI* is unusual. This might suggest that the gene cluster harboring *CPSI* came from a bacterial source (since most bacterial genes are known to have a high G+C content), but has evolved into a fungal version.

Based on these data, *CPSI* homologs may have a common ancestral gene which was acquired from a bacterial species *via* horizontal transfer and then maintained by the fungal genome *via* vertical transmission in closely related lineages.

5 In the evolution process, the genus *Cochliobolus* could also have inherited a second gene (*X*) controlling the ability to take up foreign DNA, by which its ancestor took the “alien” *CPSI*. As a result, this group of fungi is able to keep trapping genes from other organisms by additional “horizontal transfers” and giving rise to new races or even new species characterized by the ability to
10 produce unique pathogenesis factors. The direct support for this hypothesis is that both the *Tox2* locus of *C. carbonum* and the *Tox1* locus of *C. heterostrophus* are associated with large fragments of “alien” DNA (A+T-rich and highly repeated) and the same could also be true for *Tox3* controlling victorin production by *C. victoriae*, although there is yet no direct experimental evidence
15 (Ahn and Walton, 1996, *supra*; Yang et al., 1996, *supra*; Yoder et al., 1997, *supra*). In contrast to *CPSI*, these gene transfers must have occurred in the recent evolutionary past because both *Tox1* and *Tox2* loci are found only in specific isolates in the species, e.g., the acquisition of *Tox1* genes probably occurred as recently as the 1960s when race T was first identified in the field
20 (Yoder et al., 1997, *supra*).

 There are other possibilities for the evolution of *CPSI*. First, each genus mentioned above could have acquired *CPSI* independently after divergence of the lineage. But this seems less likely because this would need to happen at the same time and involve the same donor organism if the fact that the homologs
25 detected in *Cochliobolus* and *Setosphaeria* gave similar hybridization signal intensity is considered. Second, the horizontal transfer of *CPSI* could have occurred at earlier time periods such as before the divergence of Pleosporales or even the Ascomycotina. To test these hypotheses, detection of *CPSI* homologs in *Pyrenophora*, *Pleospora* and other genera must be done by either genomic
30 DNA hybridization or PCR. Based on the facts discussed here, it is not unreasonable to predict that additional *CPSI* homologs will be found in other

fungus species. Further investigation could provide a direct entry point for understanding the evolution of fungal pathogenesis to plants.

Example 5

5 Other Genes Near *Cochliobolus CPS1*

Materials and Methods

10 Construction of genomic library of *C. heterostrophus*. The cosmid SuperCosP1-11 (kindly provided by Dr. Thomas Hohn of Mycotoxin Research Unit USDA/ARS), which is a modification of the cosmid vector cosHyg1 (Turgeon et al., 1993, *supra*), was used for library construction. Genomic DNA of strain C4 (*Tox*⁺; *MAT*-2) was prepared as previously described (Yoder, 1988, *supra*) and purified by the equilibrium centrifugation in CsCl-ethidium bromide gradients (Sambrook, et al., 1989, *supra*). Three μ g of genomic DNA was partially digested with *Mbo*I using a test series of enzyme dilutions (1.5×10^{-4} -
15 1.25 units, New England Biolabs, Beverly, MA) at 37°C for 0.5 hour. DNA from the digestions which yielded fragments with an average size of 30 kb was pooled and then dephosphorylated with Calf Intestinal Alkaline Phosphatase (CIAP, GIBCO BRL Products, Gaithersburg, MD). Two μ g of CIAP-treated DNA was ligated into the *Bam*HI site of the cosmid vector that had been digested with *Xba*I
20 and treated with CIAP. Aliquots of the ligated molecules were packaged using Gigapack II Packaging Extract (Stratagene, La Jolla, CA) according to the manufacturer's recommendations. *E. coli* strain NM554 was transfected with the packaged phage particles and selected for ampicillin resistance. Approximately 1.6×10^5 independent ampicillin resistant colonies were obtained from two
25 experiments. Cosmid DNAs were made from 16 colonies and digested with *Hind*III and *Eco*RI respectively to confirm random insertions. Colonies were scraped from each of the original LB plus ampicillin plates and stored at -70°C in 25% glycerol (one plate of colonies/per tube).

30 Screening of the cosmid library. A mixture of cosmid clones from 23 stored tubes was diluted to 10^{-4} spread on ten LB plus ampicillin plates (150 X 15 mm) and incubated at 37°C overnight. Colonies (total about 1.2×10^4) were

transferred to Colony/Plaque Screen™ Hybridization Transfer Membrane (137 Mm discs, NEN™ Life Science Products, Boston, MA) and incubated at 37°C for 8 hours. Three replicates were made of each plate (one as master filter and two for probing). For hybridization, filters carrying colonies were lysed in 0.5 N NaOH, 1.5 M NaCl for 5 minutes, neutralized twice in 1 M Tris pH 7.4, 1.5 M NaCl for 5 minutes followed by 2 X SSC for 2 minutes. Filters were air dried 30 minutes then baked in a vacuum oven at 80°C for 1 hour. Duplicate filters were probed with ³²P labeled 3.4 and 3.2 kb fragments of the *CPSI* gene (cloned on p214B7 and p214S1, respectively) that were prepared by restriction enzyme digestion and purification using QIAquick Gel Extraction Kit (QIAGEN Inc., Chatsworth, CA). Hybridization was in 6 X SSC, 1 X BLOTTO (Sambrook et al., 1989) at 65°C overnight. Then filters were then washed twice for 15 minutes, 65°C in 2 X SSC, 0.1% SDS. Cosmid clones corresponding to positive areas were transferred from the master filters into a 96-well microtiter plate (Corning Costar, Cambridge, MA) and allowed to grow at 37°C overnight. Cells were then transferred onto membranes using a frogger, incubated and processed same as above. Positive clones were purified and re-tested by hybridization with the same probes as mentioned above. The isolated cosmid clones were mapped by probing cosmid DNA digested with several enzymes with the labeled 3.4 and 3.2 kb *CPSI* fragments separately.

DNA manipulations and sequencing. Cosmid DNA was prepared using standard protocols (Sambrook, et al., 1989, *supra*). Restriction enzyme digestions, gel electrophoresis, gel blot analysis, primer design, DNA sequencing and sequence analysis were done as described above. To facilitate sequencing, three deletion constructs were made by digestion of the original cosmid clones (Table 10) with restriction enzymes that do not cut the cosmid vector, followed by religation (Table 10). Sequencing of each cosmid clone was initiated with vector-specific and *CPSI* (or *TESI*)-specific primers. Subsequently, sequences were extended by designing new primers to the previously sequenced region (Table 11).

Results

Characterization of two overlapping cosmid clones. Two cosmid clones, C4L6582 and C4L7296, were isolated by screening the library (Table 10). Gel blot analysis indicated that both cosmid clones span the vector insertion site in the REMI mutant and contain the cloned *CPSI* and *TESI* sequences described

5 above. Sequence obtained using a primer to the region immediately flanking the insertion site is the same as that in the tagged DNA recovered from the REMI mutant, confirming that no deletions or chromosome rearrangements occurred at the tagged site. Two cosmids overlap each other in a 27.9 kb region. C4L7296 (37.2 kb) carries a 30.9 kb genomic insert which hybridized to both 3.4 kb and

10 3.2 kb *CPSI* fragments. Restriction mapping and sequencing confirmed that this insert contains the entire *TESI* sequence and most of the *CPSI* sequence (4.4 out of 5.4 kb). C4L6582 (37.7 kb) carries a 31.4 kb insert that also includes the entire *TESI* sequence but only 1.1 kb of the N-terminal encoding sequence of *CPSI*. Both inserts lack the C-terminal region of *CPSI*; their 3' end is ligated to

15 the T3 end of cloning site in SuperCosP1-11. Attempts to sequence using the T7 primer were unsuccessful, presumably because the T7 end, which is close to one of the *cos* sites on SuperCosP1-11 was disrupted during the packaging process.

Table 10. Cosmid and plasmid clones used in this study

| | Clones | Length (kb) | Characteristics | Reference |
|----|--|----------------|---|-----------------|
| 5 | Super- al., CosP1-11 | 6.9 | Cosmid vector for library construction containing the 2.5 kb <i>HindIII</i> - <i>SalI</i> fragment from pH1S carrying <i>hygB</i> gene fused to <i>C. heterostrophus</i> promoter 1. | Horwitz et 1997 |
| | pUCATPHN | 4.6 | Cloning vector derived from pUCATPH. | This study |
| 10 | C4L6582 | 37.7 | A cosmid clone with a 31.4 kb insert isolated from screening the library. Includes 4.0 kb region p214B7. | This study |
| | C4L7296 | 37.2 | A cosmid clone with a 30.9 kb insert isolated from screening the library. Includes 6.3 kb region p214B7+p214S1. | This study |
| 15 | p6582dH | 10.9 | A deletion (28.8 kb) construct derived from digestion of C4L6582 with <i>HindIII</i> . | This study |
| | p6582dS | 21.1 | A deletion (16.6 kb) construct derived from digestion of C4L6582 with <i>SacI</i> . | This study |
| 20 | p7296dX | 9.0 | A deletion (28.2 kb) construct derived from digestion of C4L7296 with <i>XhoI</i> . | This study |
| | pDXPS* | 13.6 | Ligation of 7296dX digested with <i>XhoI</i> to the <i>SalI</i> -digested pUCATPHN. | This study |
| 25 | pDXPSH* | 6.5 | A plasmid derived from pDXPS by <i>HindIII</i> digestion and religation of a 6.5 kb <i>HindIII</i> fragment containing the entire pUCATPHN sequence flanked by 1.2 kb of the 5' end of CPS1 and 0.5 kb 3' end of C4L7296 sequence | This study |
| 30 | * Designed for deletion of the 28.2 kb of genomic region (= deleted from p7296dX, including 3.6 kb <i>CPSI</i> N-terminal encoding sequence) but transformation of wild type was unsuccessful. | | | |

Table 11. Primers used for sequencing genomic DNA on C4L7296 and C4L6582

| 5 | Name ^a | Position ^b | Sequence ^c | Template ^d | Origin |
|----|-------------------|-----------------------|-----------------------|-----------------------|-----------|
| | F-I | | | | |
| | 214RP7 | | SEQ ID NO:148 | A | p214B7 |
| | 1. RP8 | 4940 | SEQ ID NO:149 | A | 7296RP |
| | 2. RP9 | 592 | SEQ ID NO:150 | A | 7296RP8 |
| 10 | 3. RP10 | 4124 | SEQ ID NO:151 | A | 7296RP9 |
| | 4. RP11 | 3790 | SEQ ID NO:152 | A | 7296RP10 |
| | 5. RP12 | 3424 | SEQ ID NO:153 | A | 7296RP11 |
| | 6. RP13 | 2970 | SEQ ID NO:154 | A | 7296RP12 |
| | 7. RP14 | 2362 | SEQ ID NO:155 | A | 7296RP13 |
| 15 | 8. RP15 | 1764 | SEQ ID NO:156 | A | 7296RP14 |
| | 9. RP16 | 1169 | SEQ ID NO:157 | A | 7296RP15 |
| | 10. RP17 | 647 | SEQ ID NO:158 | A | 7296RP16 |
| | F-II | | | | |
| | 214RP2 | | SEQ ID NO:159 | B | p214B7 |
| 20 | 11. SRP1 | 3095 | SEQ ID NO:160 | A | 6582dSRP2 |
| | 12. SRP2 | 2755 | SEQ ID NO:161 | A | 7296dSRP1 |
| | 13. SRP3 | 2366 | SEQ ID NO:162 | A | 7296dSRP2 |
| | 14. SRP4 | 2008 | SEQ ID NO:163 | A | 7296dSRP3 |
| | 15. SRP5 | 1555 | SEQ ID NO:164 | A | 7296dSRP4 |
| 25 | 16. SRP6 | 1187 | SEQ ID NO:165 | A | 7296dSRP5 |
| | 17. SRP7 | 647 | SEQ ID NO:166 | A | 7296dSRP6 |
| | 18. SFP1 | 3321 | SEQ ID NO:167 | A | 6582dSRP2 |
| | 19. SFP2 | 3660 | SEQ ID NO:168 | A | 7296dSFP1 |
| | 20. SFP3 | 3969 | SEQ ID NO:169 | A | 7296dSFP2 |
| 30 | 21. SFP4 | 4345 | SEQ ID NO:170 | A | 7296dSFP3 |
| | 22. SFP5 | 4724 | SEQ ID NO:171 | A | 7296dsFP4 |
| | 23. SFP6 | 5137 | SEQ ID NO:172 | A | 7296dSFP5 |
| | 24. SFP7 | 694 | SEQ ID NO:173 | A | 7296dSFP6 |

| | | | | | |
|-------|----------|------|---------------|---|------------|
| F-III | | | | | |
| | TrpC | | SEQ ID NO:174 | C | pUCATPH |
| | 214FP6 | | SEQ ID NO:175 | D | p 214S1 |
| | 25. CFP1 | 463 | SEQ ID NO:176 | A | pDXPSTrpC |
| 5 | 26. CFP2 | 903 | SEQ ID NO:177 | A | 7296pUCFP1 |
| | 27. CFP3 | 1334 | SEQ ID NO:178 | A | 7296pUCFP2 |
| | 28. CFP4 | 1910 | SEQ ID NO:179 | A | 7296pUCFP3 |
| | 29. CFP5 | 2491 | SEQ ID NO:180 | A | 7296pUCFP4 |
| F-IV | | | | | |
| 10 | 214B7RP5 | | SEQ ID NO:181 | E | p214B7 |
| | 30. HRP1 | 592 | SEQ ID NO:182 | F | 6582dHRP5 |
| | 31. HFP1 | 763 | SEQ ID NO:183 | F | 6582dHRP5 |

^a “RP” indicates reverse primer; “FP” indicates forward primer. Primers designed to genomic DNA on the cosmid clones are numbered in order. Primers 1-10 are preceded by “7296”; 11-24 by “7296d”; 25-29 by “7296pU” and 30-31 by “6582d”.

^b Primer position corresponds to position in the genomic sequences of each fragment.

^c Each primer sequence is given in the 5' to 3' direction.

^d Cosmids or plasmids used for sequencing reactions. A = C4L7296; B = 6582dS; C = pDXPS; D = pDXPSH; E = 6582dH; F = C4L6582.

Sequencing of C4L7296. A total of 27.4 kb additional genomic sequence 5' of *TES1* was cloned. Four fragments with totaling 16.9 kb (60%) were sequenced, three of which were sequenced using C4L7296 as template. Sequencing of Fragment I (F-I, 5.3 kb) began with primer 214B7RP7 (which matches the 5' end of *TES1*), then was followed by sequencing with primers designed to previously determined sequences. Fragment II (F-II, 6.9 kb) was started using primers to sequences flanking the *SacI* site previously determined by sequencing the deletion construct 6582dS (see Table 10) and subsequently extended in both directions. Sequence of Fragment III (F-III, 3.2 kb) was obtained in a complicated manner as part of the attempt to create a deletion construct for transformation. The first part of the sequence was obtained from the clone pDXPS derived from deletion construct 7296dX (Table 10) using the

TrpC primer and the sequence was extended to the 3' end using C4L7296 as template. A 200 bp region at the 5' end of FIII was obtained from a pDXPS derived clone, pDXPSH (Table 10), using a *CPS1*-specific primer 214S1FP6.

5 Sequencing of C4L6582. This clone contains 2.8 kb additional genomic DNA extending into the region to the left end of C4L7296. The deletion clone 6582dH (Table 10) was used to initiate sequencing of Fragment IV (F-IV, 1.5 kb) using a *TES1*-specific primer 214B7RP5 followed by one step of sequence extension in both 3' and 5' direction on C4L6582.

10 Identification of open reading frames in the sequenced region. Eleven open reading frames (ORF) were identified in the four sequenced fragments (Table 12). These ORFs are all relatively small (0.3-2.3 kb). Five ORFs contain putative introns with typical fungal characteristics (Table 13). ORF12, ORF10, ORF14, ORF5 and ORF8 are transcribed in one direction; others are transcribed in the opposite direction. ORF6 and ORF7 (in F-II) overlap and are transcribed
15 in the same direction. ORF14 and ORF9 (in F-III), ORF3 and ORF8 (in F-I) also overlap but are transcribed to the opposite directions. Most ORFs have G+C content between 50-55% in the normal range for most fungal genes with the two exceptions: ORF (0.3 kb) in the 5' end of F-III has a G+C content of 63.6%; ORF14 (0.7 kb, located 1.0 kb downstream of ORF10) has a G+C content
20 56.9%. Both ORFs are located in a G+C-rich (about 58.0%) region in F-III (positions 300-800 and 1240-2040, respectively).

Database searches suggested that three ORFs (ORF3, ORF7 and ORF11) as well as *CPS1* and *TES1* encode homologs of known proteins (see below) and others encode, if anything, proteins with unknown functions (Table 12). ORF 17
25 (SEQ ID NO:48) encodes an iron reductase (SEQ ID NO:49) and ORF15 (SEQ ID NO:55) encodes a permease/MFS transporter (SEQ ID NO:56). Figure 9A shows the results of a BLAST search with SEQ ID NO:49 and Figure 10 shows the results of a BLAST search with the polypeptide encoded by SEQ ID NO:55.

Table 12. Open reading frames (ORFs) identified in sequenced genomic regions of C4L7296 and C4L6582

| | Region ^a | ORF ^b | Size (kb) | No. of introns | G+C (%) | Putative Function |
|----|---------------------|--------------------------|-----------|----------------|---------|---------------------------|
| 5 | | | | | | |
| | F-I' | <u>ORF1</u> ^d | 5.4 | 3 | 51.5 | <u>Peptide synthetase</u> |
| | F-I' | <u>ORF2</u> ^d | 1.1 | 1 | 55.5 | <u>Thioesterase</u> |
| | F-I | <u>ORF3</u> | 1.8 | 3 | 50.0 | <u>DNA-binding</u> |
| 10 | F-I | ORF8 | 0.5 | 0 | 55.2 | unknown |
| | F-I | <u>ORF11</u> | 1.9 | 0 | 52.6 | CoA transferase |
| | F-II | ORF5 | 2.3 | 1 | 54.1 | unknown |
| | F-II | ORF6 | 0.5 | 0 | 51.6 | unknown |
| | F-II | <u>ORF7</u> | 1.7 | 1 | 52.0 | <u>Decarboxylase</u> |
| 15 | F-III | ORF9 | 0.7 | 0 | 54.2 | unknown |
| | F-III | ORF10 | 0.3 | 0 | 63.6 | unknown |
| | F-III | ORF13 | 0.8 | 1 | 53.6 | unknown |
| | F-III | ORF14 | 0.7 | 0 | 56.9 | unknown |
| 20 | F-IV | ORF12 | 1.2 | 1 | 49.2 | unknown |

^a F-I'= Genomic DNA

^b The positions of ORF3-ORF14 and 17 in the sequenced fragment is indicated; ORFs corresponding to known proteins are underlined.

^c The characteristics of putative introns are given in Table 12.

25 ^d Characterized as *CPSI* and *TESI*

Table 13. Characteristics of putative introns in ORFs identified in sequenced genomic regions on cosmids C4L7296 and C4L6582

| | ORF | Intron | Size (bp) | Location ^a | 5'Border | 3'Border | Branch site |
|----|-----------|--------|-----------|-----------------------|------------------------------------|----------------------------------|----------------------|
| 30 | | | | | | | |
| | ORF3 | I | 64 FI | 5094-5031 | GTACGT | TAG | CGCTGAC |
| | | II | 46 FI | 5006-4961 | GTGAGT | TAG | AGCTAAG |
| 35 | | III | 46 FI | 4477-4432 | GTACGT | CAG | AGCTGAC |
| | ORF5 | I | 48 FII | 3477-3524 | GTATGT | TAG | TGCTAAC |
| | ORF7 | I | 114 FII | 2307-2194 | GTGTGC | CAG | ATCTAAC |
| | ORF13 | I | 51 FIII | 2742-2692 | GTGCGT | CAG | TACTGAT |
| | ORF12 | I | 47 FIV | 1007-1053 | GTAAGT | TAG | GATTGAC |
| 40 | | | | | | | |
| | Consensus | | | | GT ^A / _G YGT | T ^T / _C AG | NRCTAAC ^b |

^a Number of the fragment followed by the position of the first and last nucleotide of the intron with respect to the total sequence.

^b Y = Pyrimidine (T or C); R = purine; N = purine or pyrimidine.

Discussion

Two cosmids define a large gene cluster. The *C. heterostrophus CPSI* gene was cloned by identification of genomic DNA fragments recovered from the tagged site in a mutant generated using REMI insertional mutagenesis. Characterization of two overlapping cosmid clones in this study has proved that no deletions or chromosome rearrangements are associated with the gene tagging event, because both cosmids carry the same fragment which span the REMI insertion site and the nucleotide sequence in this region is the same as that of recovered genomic DNA from the tagged site. This undoubtedly clarifies the identity of *CPSI*, which is the major biosynthetic gene. Mapping and sequencing of the two cosmids extended the sequence by 27.4 kb from the previously cloned fragment, leading to the characterization of 38.7 kb of contiguous genomic DNA, the largest genomic region analyzed so far in *C. heterostrophus*. In addition to *CPSI* and *TESI*, sequence analysis of this region revealed at least 11 open reading frames; three of them, designated as *DBZ1*, *CAT1* and *DEC2*, respectively, apparently encode functional proteins (Table 13). The tight linkage of these genes suggests that they may be involved in the same pathway.

In filamentous fungi, in some cases, genes in pathways for biosynthesis of secondary metabolites are dispersed on different chromosomes, e.g., the cephalosporin C pathway genes in *Acremonium chrysogenum* (Mathison et al., 1993, *supra*) and the melanin pathway genes in *Colletotrichum lagenarium* (Kubo et al., 1996, *supra*). In other cases, tightly linked genes are usually found to be functionally related to a common pathway. This clustering organization has been exemplified by the sterigmatocystin pathway genes of *Aspergillus nidulans*, in which 25 coordinately regulated transcripts are found in a 60 kb genomic region (Brown et al., 1996) and the trichothecene pathway genes of *Fusarium sporotrichioides*, in which 9 genes are clustered in a 25 kb region and 8 of them have been shown to be required for the pathway function (Hohn et al., 1995). The genes involved in biosynthesis of certain fungal peptides are also

found as clusters. The tight linkage between *CPSI* and these additional genes might reveal the presence of a novel secondary metabolite pathway in *C. heterostrophus*. In this pathway, *CPSI* is the major structural gene since it encodes a large multifunctional enzyme with all catalytic activities required for synthesis of a secondary metabolite, presumably a peptide phytotoxin; other genes may carry out different functions required for coordinate operation of the pathway, such as regulation, posttranslational modification or substrate processing as discussed below.

Significance of the *CPSI* gene cluster. Both functional and structural analyses strongly support the hypothesis that the *CPSI* gene cluster controls a novel biosynthetic pathway. Pathway genes have been studied only in a few filamentous fungi mainly for industrial purposes (Keller et al., 1997, *supra*). For plant pathogenic fungi, little is known about pathway genes for fungal pathogenesis. In *C. heterostrophus*, recent cloning of two *Tox1* genes *PKSI* (Yang et al., 1996, *supra*) and *DECI* (Rose et al., 1996, *supra*) have contributed to a breakthrough in understanding the molecular mechanism for biosynthesis of T-toxin, a virulence determinant in the fungus/corn interaction. But further identification of related pathway genes has been unsuccessful because the two genes are located on different chromosomes and each is embedded in A+T-rich DNA (Yoder et al., 1997, *supra*). In contrast, the *CPSI* cluster provides a good opportunity to explore a pathogenesis pathway.

First, it resides in a "normal" sequence region. G+C content of a 50-55% is found in most of the cloned sequences and no A+T-rich DNA is associated with either end of the cloned region. This would facilitate cloning of additional pathway genes by further chromosome walking, by screening of cosmid libraries or the targeted integration and plasmid rescue. Second, it contains a regulatory gene (*DBZI*) which is presumably linked to a signal transduction pathway. Isolation of genes that interact with *DBZI* could reveal novel factors mediating the molecular communication between fungal pathogen and the host plant. Further characterization of *DBZI* (along with position-specific disruption or deletion) would be also helpful in determining the limit of the gene cluster,

because tightly linked genes involved in a common pathway are often coordinately regulated by the same regulatory factor (Keller et al., 1997, *supra*). Finally, *CPS1* genes are found in both race T and race O, and its homologs are also found in other *Cochliobolus* species. Presence of high G+C content may
5 imply that these genes evolved from a bacterial ancestor and the conservation in these fungi may correlate with the phytopathogenic function of the gene products encoded by the *CPS1* cluster. Further investigation of this cluster should provide insights into the evolution of general pathogenicity factors among this group of fungi.

10 ORF17 is an iron reductase (SEQ ID NO:49) and ORF15 is a permease/MFS transporter (SEQ ID NO:56). Ferric reductases are a group of enzymes found in bacteria, fungi, plants and animals that are responsible for reduction of ferric iron to ferrous iron, an absorptive form used by the organism. They have been well studied in *S. cerevisiae*, *C. albicans* and *H. capsulatum* and
15 the like. The yeast FER1 has been expressed in tobacco (Oki et al., 1999).

Previous studies have shown that FER genes could be important pathogenic determinants. Timmerman and Woods have proposed that in *H. capsulatum* FER could play critical roles in the acquisition of iron in three different ways: from inorganic or organic ferric salts, from host Fe(III) binding
20 proteins (transferrin and the like), and from siderophores produced by the fungus itself (to reduce and release the iron chelated by the siderophore molecules).

On the other hand, iron sequestration in response to microbial infection has been demonstrated to be a host defense mechanism. The infection-related iron acquisition system in the pathogen can be considered to be an important
25 mechanism against host defense and for a successful colonization by the pathogen in the host cells. This could be a general mechanism for all pathogenic fungi.

CPS1 may encode an enzyme which is responsible for biosynthesis of a novel siderophore with unusual amino acid, hydroxyl acid and architecture. The
30 CPS1 siderophore can compete with the host for iron acquisition when the fungus enters its host cells where the iron is limited due to host sequestration. In

particular, for root pathogens such as *C. victoriae*, sequestration may be stronger in the root surface. This could explain why the *cps1* mutant showed drastically reduced virulence. The FER1 could be required to release iron from the CPS1 siderophore which explains its location near the *CPS1* gene. Moreover, fungal
5 strains could be cultured in iron-limiting conditions because CPS1, and likely other genes in the cluster maybe turned on only during conditions of iron depletion.

All publications, patents and patent applications are incorporated herein
10 by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from
15 the basic principles of the invention.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a fungal nucleic acid segment
which encodes a polypeptide which is substantially similar to a
5 polypeptide encoded by a nucleic acid sequence comprising an open
reading frame comprising SEQ ID NO:46, SEQ ID NO:48, or SEQ ID
NO:55, or the complement thereof.
2. An isolated polynucleotide comprising a fungal nucleic acid segment
10 which is substantially similar to a nucleic acid sequence comprising an
open reading frame comprising SEQ ID NO:46, SEQ ID NO:48, SEQ ID
NO:55, or the complement thereof.
3. An isolated polynucleotide comprising a fungal nucleic acid segment
15 which hybridizes under stringent hybridization conditions to SEQ ID
NO:46, SEQ ID NO:48, SEQ ID NO:55, or the complement thereof.
4. The isolated polynucleotide of claim 1, 2 or 3 which consists of SEQ ID
NO:46, SEQ ID NO:48 or SEQ ID NO:55 of the complement thereof.
20
5. The isolated polynucleotide of claim 1, 2 or 3 wherein the nucleic acid
segment is from *Ascomycota*.
6. The isolated polynucleotide of claim 1, 2 or 3 wherein the nucleic acid
25 segment is from a pathogenic fungus.
7. The isolated polynucleotide of claim 1 wherein the nucleic acid segment
encodes a polypeptide having at least 80% identity to a polypeptide
comprising SEQ ID NO:47, SEQ ID NO:49 or SEQ ID NO:56.
30

8. The isolated polynucleotide of claim 1 wherein the nucleic acid segment encodes a polypeptide having at least 90% identity to a polypeptide comprising SEQ ID NO:47, SEQ ID NO:49 or SEQ ID NO:56.
- 5 9. An isolated polypeptide encoded by the polynucleotide of any one of claims 1 to 8.
10. An expression cassette comprising a promoter operably linked to the polynucleotide of any one of claims 1 to 8.
- 10 11. A recombinant vector comprising the polynucleotide of any one of claims 1 to 8 wherein the vector is capable of being stably transformed into a host cell.
- 15 12. The vector of claim 11 wherein the polynucleotide is operably linked to a promoter operable in a eukaryotic host cell.
13. The expression cassette of claim 10 or vector of claim 11 wherein the polynucleotide is in sense orientation.
- 20 14. The expression cassette of claim 10 or vector of claim 11 wherein the polynucleotide is in antisense orientation.
15. The vector of claim 11 wherein the polynucleotide is operably linked to a promoter operable in a prokaryotic host cell.
- 25 16. A host cell comprising the expression cassette of claim 10.
17. A host cell comprising the vector of claim 11.
- 30

18. The host cell of claim 16 or 17 which is selected from the group consisting of bacteria, yeast, plant and mammal.
19. A method for identifying an agent having fungicidal or mycocidal activity, comprising:
- 5 a) contacting a fungus with an agent that binds to the polypeptide of claim 9; and
- b) identifying an agent having fungicidal or mycocidal activity.
- 10 20. An agent identified by the method of claim 19.
21. A method for identifying an inhibitor of a polypeptide, comprising:
- a) contacting a host cell which expresses a polypeptide encoded by the polynucleotide of any one of claims 1 to 8 with an agent; and
- 15 b) identifying an agent that inhibits the activity of the polypeptide.
22. An agent identified by the method of claim 21.
23. A method of inhibiting the growth or pathogenicity of a fungus, comprising contacting the fungus with the agent of claim 20 or 22 in an amount sufficient to inhibit the growth or pathogenicity of the fungus.
- 20 24. A method for identifying an agent having fungicidal or mycocidal activity, comprising:
- 25 a) contacting a fungus with an agent that inhibits the activity of the polypeptide of claim 9; and
- b) identifying an agent having fungicidal or mycocidal activity.
25. A method for identifying an agent that modulates a polypeptide associated with pathogenicity of a fungus, comprising:
- 30

- a) contacting a fungus with an agent that binds the polypeptide of claim 9; and
- b) identifying an agent that modulates the pathogenicity of the fungus.
- 5
26. A method for identifying an agent that modulates the pathogenicity of a fungus, comprising:
- a) contacting a fungus with an agent that inhibits the activity of the polypeptide of claim 9; and
- 10 b) identifying an agent that modulates the pathogenicity of the fungus
27. A method of identifying agents that alter the phenotype of a fungal pathogen or mycogen, comprising:
- 15 a) contacting an agent to be tested with one or more cells of a fungal pathogen or mycogen which comprises a nucleotide sequence encoding a polypeptide that is substantially similar to SEQ ID NO:47, SEQ ID NO:49, or SEQ ID NO:56; and
- 20 b) detecting or determining whether the agent selectively modulates expression or function or metabolic pathways associated with the polypeptide, thereby altering a phenotype of the cells relative to cells not contacted with the agent.
28. The method of claim 27 wherein the polypeptide is associated with virulence or pathogenicity.
- 25
29. The method of claim 27 wherein the agent alters the activity of the polypeptide.
- 30 30. The method of claim 27 further comprising identifying an agent having fungicidal, mycocidal or anti-pathogenic activity.

31. The method of claim 27 wherein cellular growth is detected or determined.
- 5 32. The method of claim 27 wherein the activity of the polypeptide is detected or determined.
33. The method of claim 27 wherein virulence is detected or determined.
- 10 34. The method of claim 27 wherein the pathogen expresses the polypeptide.
35. The method of claim 27 wherein the pathogen does not express the polypeptide.
- 15 36. A method of identifying agents that alter the phenotype of a fungal pathogen or mycogen, comprising
- a) contacting an agent to be tested with one or more cells of a fungal pathogen or mycogen wherein the cells have a mutation in a nucleic acid sequence corresponding to the polynucleotide according to any one of claims 1 to 8 which mutation results in overexpression or underexpression of the encoded polypeptide;
- 20 b) detecting or determining whether the agent selectively modulates expression or function or metabolic pathways associated with the polypeptide, thereby altering a phenotype of the cells relative to one or more wild type cells not contacted with the agent.
- 25 37. The method of claim 27 or 36 wherein the pathway is associated with the production of a toxin or siderophore.
- 30 38. The method of claim 27 or 36 wherein the pathway is associated with iron metabolism, uptake or absorption.

39. The method of claim 27 or 36 wherein the pathway is associated with growth, virulence or pathogenicity.
40. An isolated antibody which specifically binds to the polypeptide of claim 9.
41. The antibody of claim 40 which is a monoclonal antibody.
42. The antibody of claim 40 which is a polyclonal antibody.
43. The method of claim 19, 23, 24, 25, 26, 27 or 36 wherein the fungus is a recombinant fungus.
44. The method of claim 43 wherein the fungus comprises a recombinant DNA molecule which encodes the polypeptide.
45. The method of claim 44 wherein the recombinant DNA molecule is overexpressed.
46. The method of claim 44 wherein the fungus comprises an antisense recombinant DNA molecule for the polypeptide.
47. The method of claim 44 wherein the genome of the fungus is disrupted so that the endogenous gene which encodes the polypeptide is not expressed.
48. A therapeutic method comprising: administering to an animal suspected of being infected with a fungal pathogen an effective amount of the agent of claim 19 or 22.

49. A method to prevent or inhibit infection of an animal or plant by a fungal pathogen, comprising: administering to the animal or plant an effective amount of the agent of claim 19 or 22 for a time and under conditions sufficient to inhibit or prevent fungal growth or reproduction.
- 5
50. The method of claim 51 or 52 wherein the animal is a human.
51. The method of claim 51 or 52 wherein the agent is topically administered.
- 10
52. A nucleic acid sequence of a polynucleotide of any one of claims 1 to 8.
53. The nucleic acid sequence of claim 52 which is stored on a computer readable medium.
- 15
54. An amino acid sequence of a polypeptide of claim 9.
55. The amino acid sequence of claim 54 which is stored on a computer readable medium.
- 20
56. The method of claim 48 or 49 wherein the animal is immunocompromised.
57. The method of claim 48 or 49 wherein the animal has
- 25 Coccidioidomycosis.
58. The method of claim 48 or 49 wherein the animal is subjected to immunosuppressive therapy.
- 30
59. The method of claim 48 or 49 wherein fungal iron metabolism is inhibited.

60. The method of claim 49 wherein the agent is administered to a plant.
61. The method of claim 60 wherein the agent is administered by spraying.
- 5
62. A transformed plant, the genome of which expresses a chimeric DNA molecule which encodes a gene product which confers resistance or tolerance to the plant to a fungal pathogen by inhibiting fungal iron metabolism or siderophore production.
- 10

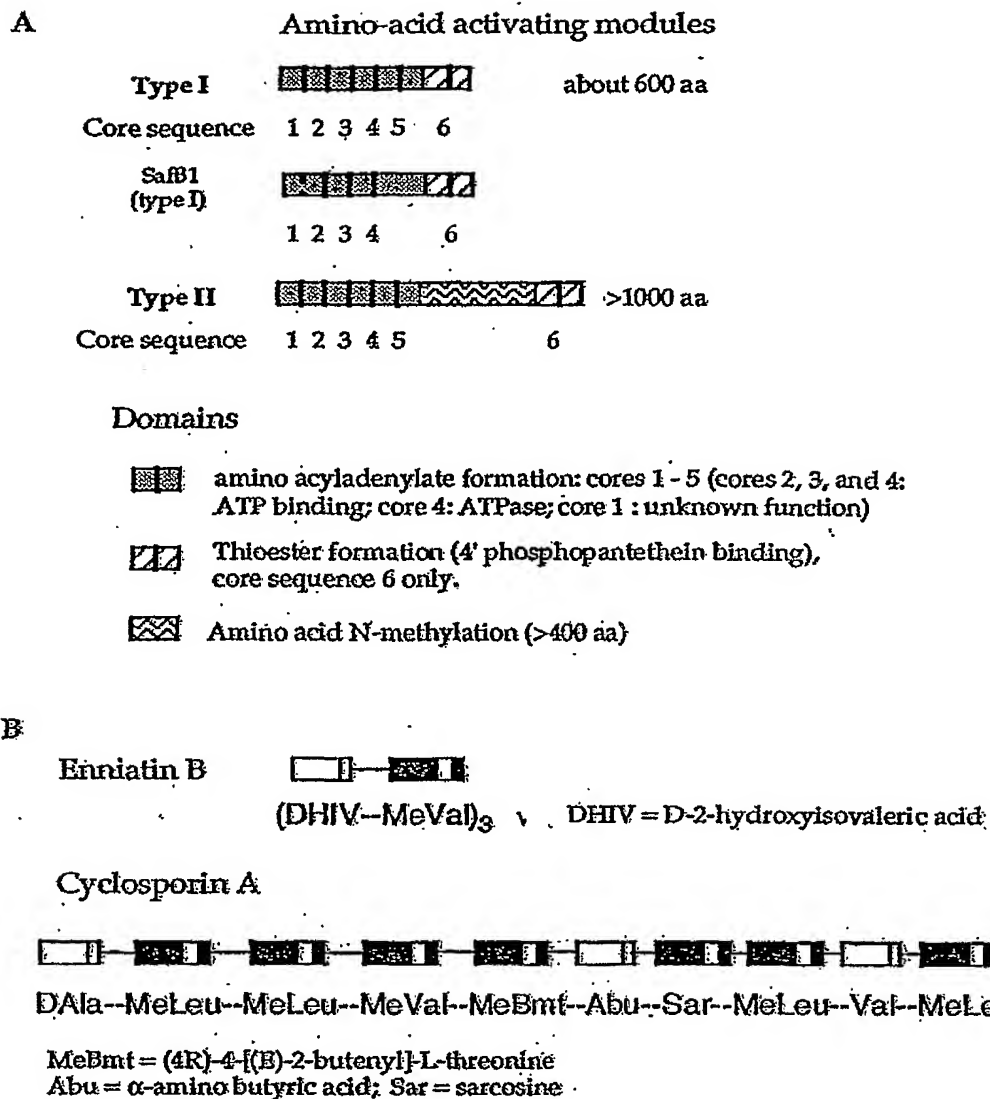


Figure 1

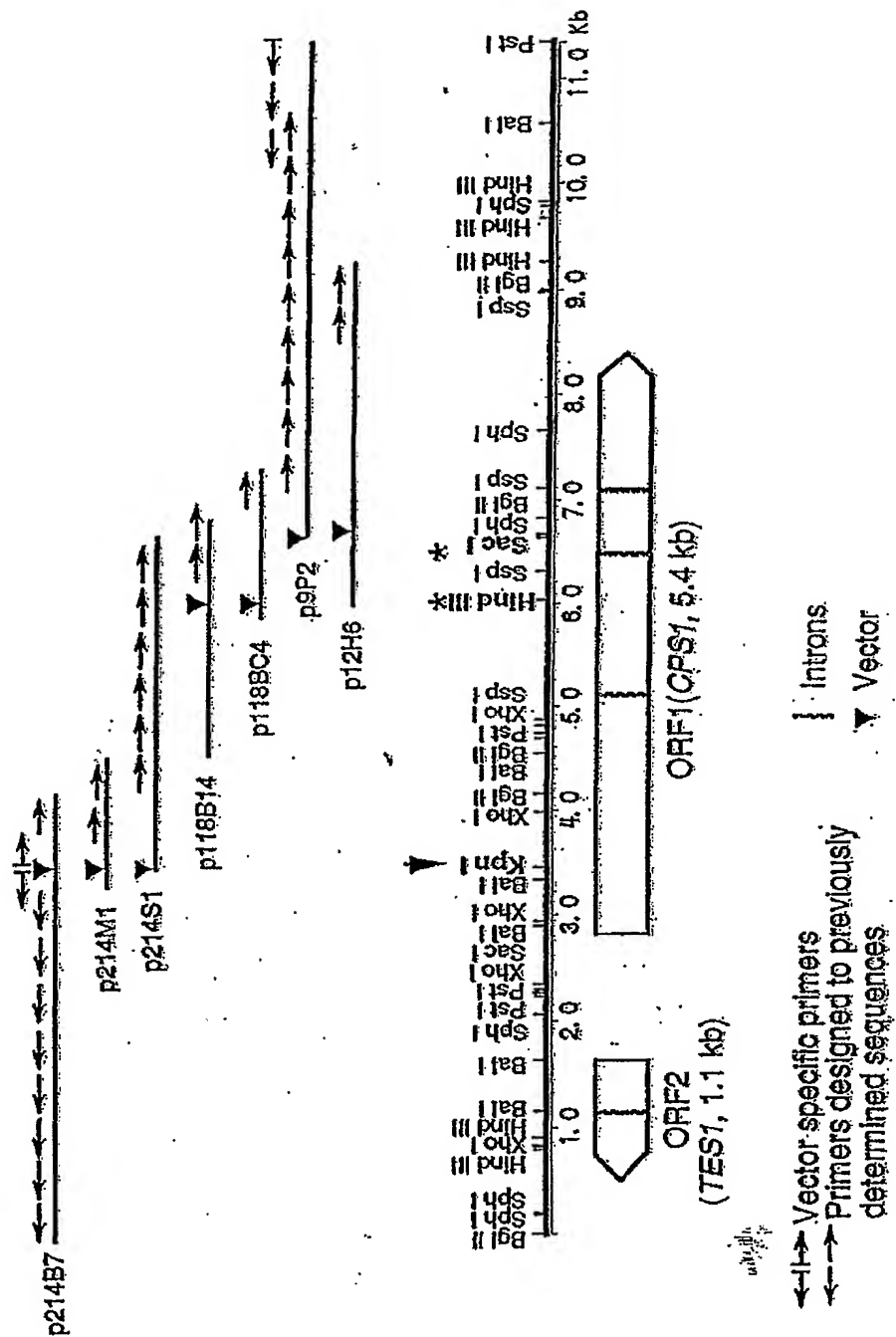


Figure 2

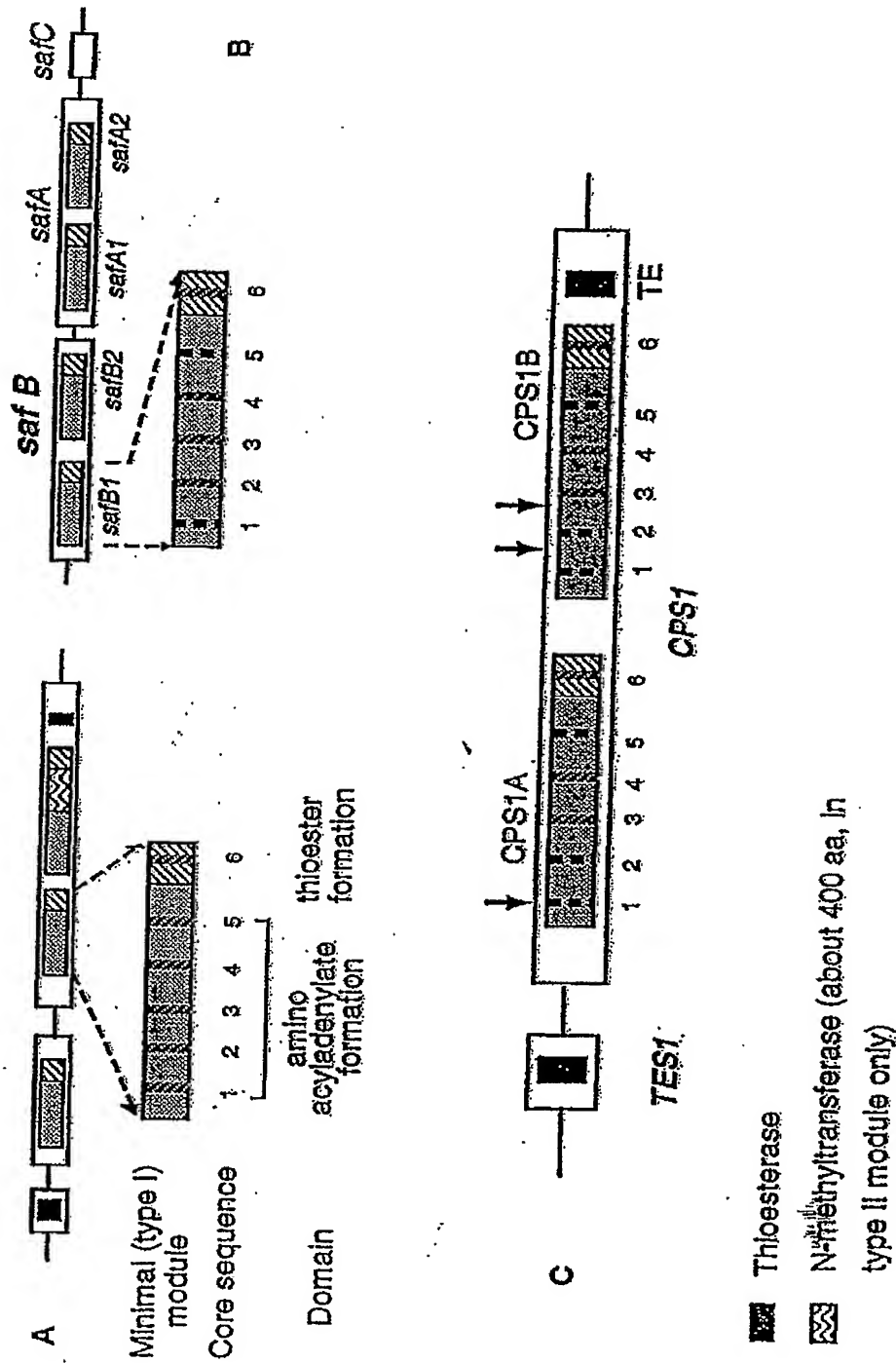


Figure 3A-C

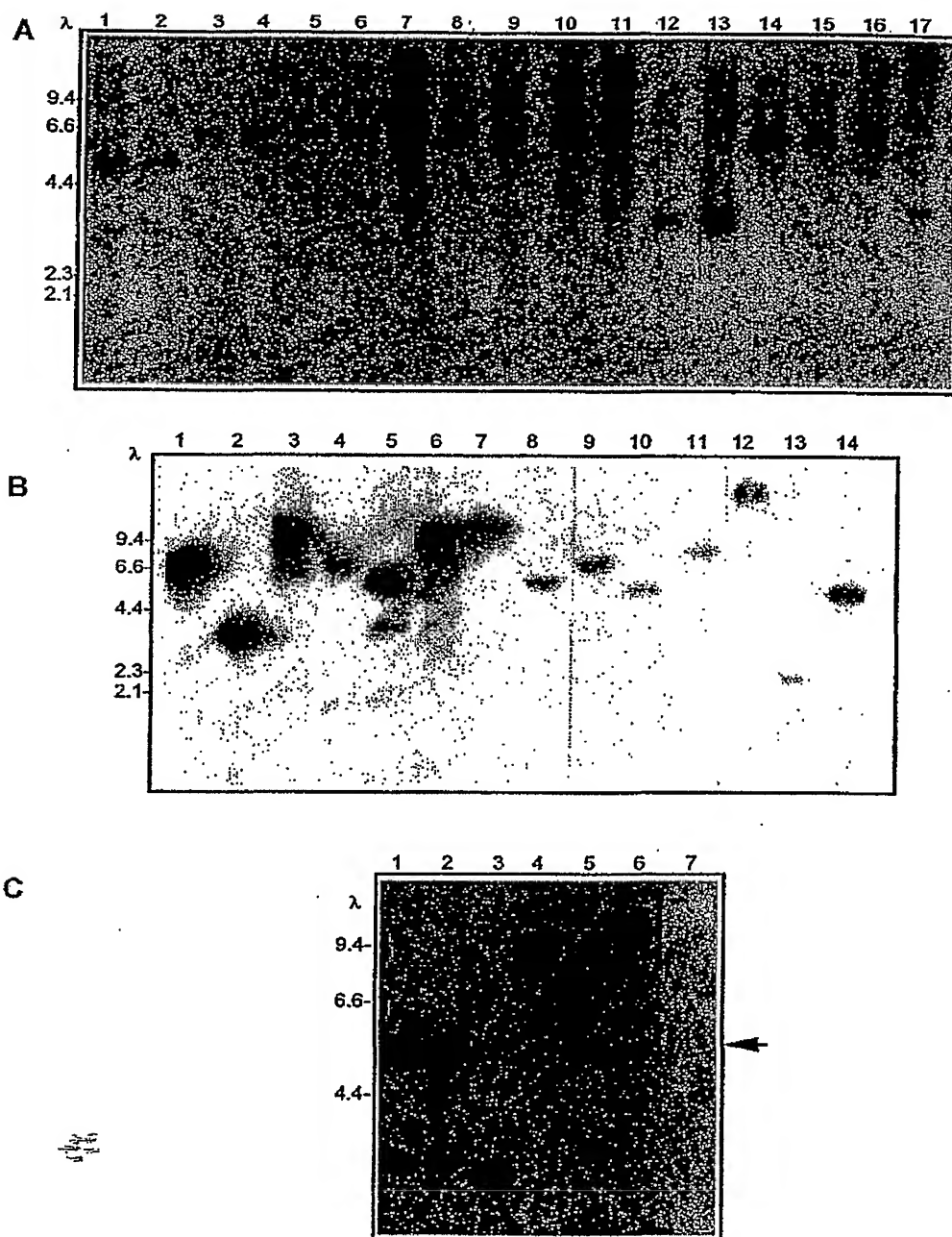


Figure 21

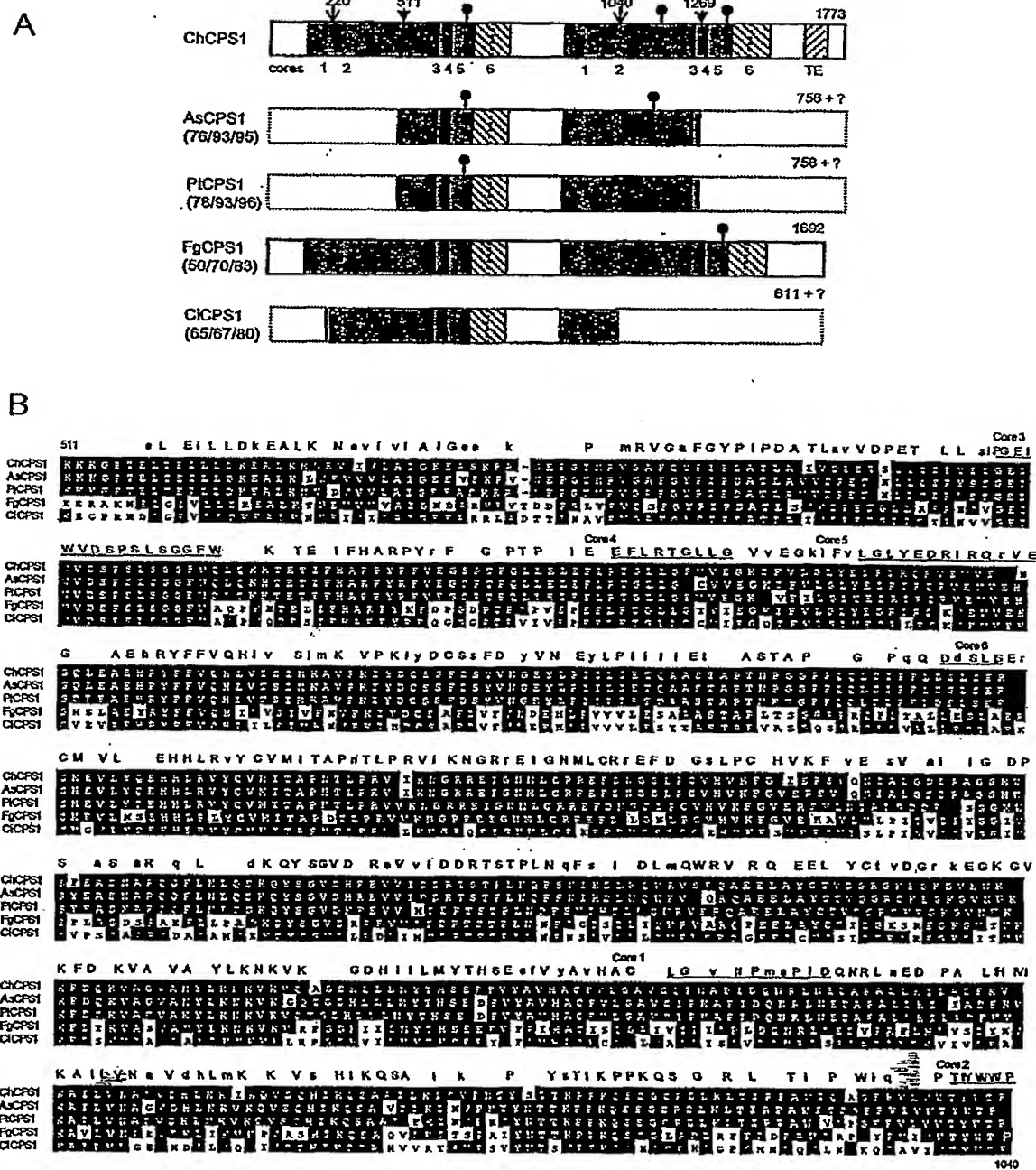
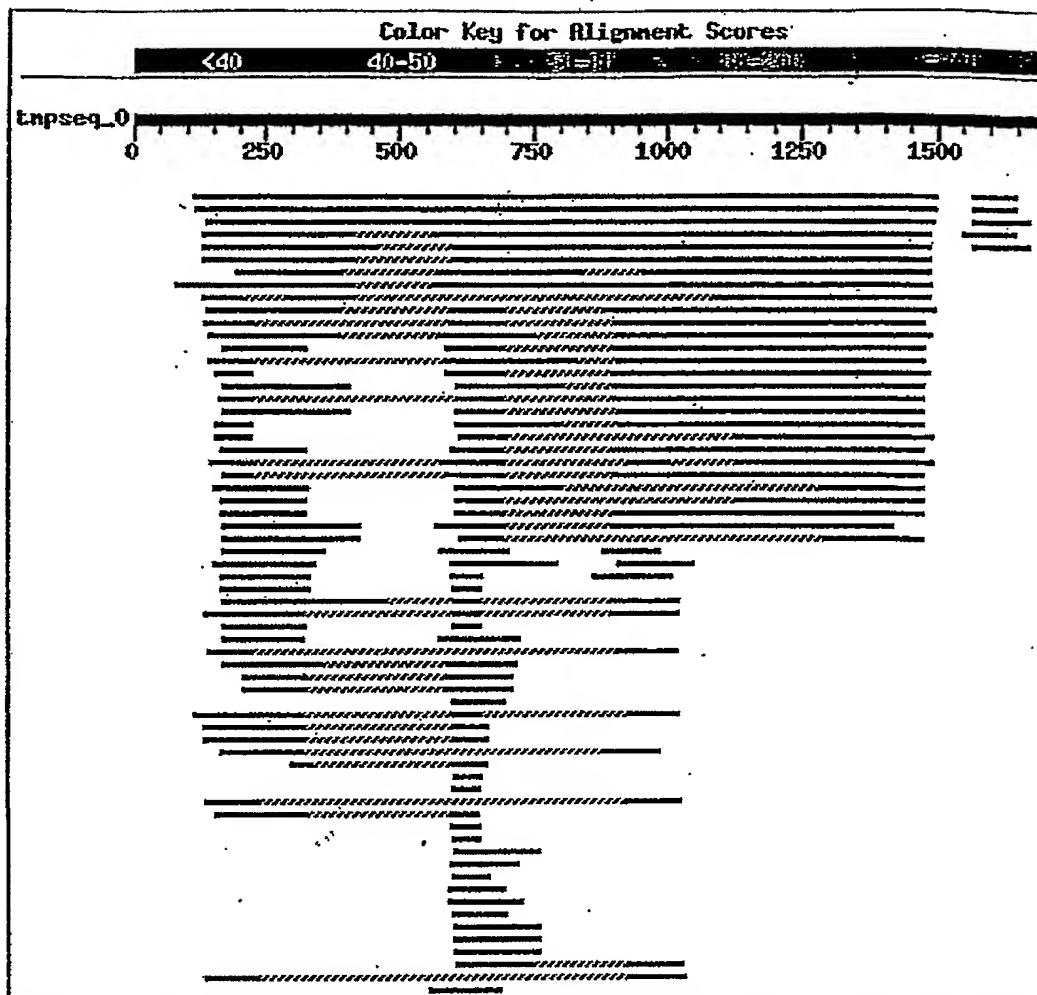


Figure 5A-B



Sequences producing significant alignments:

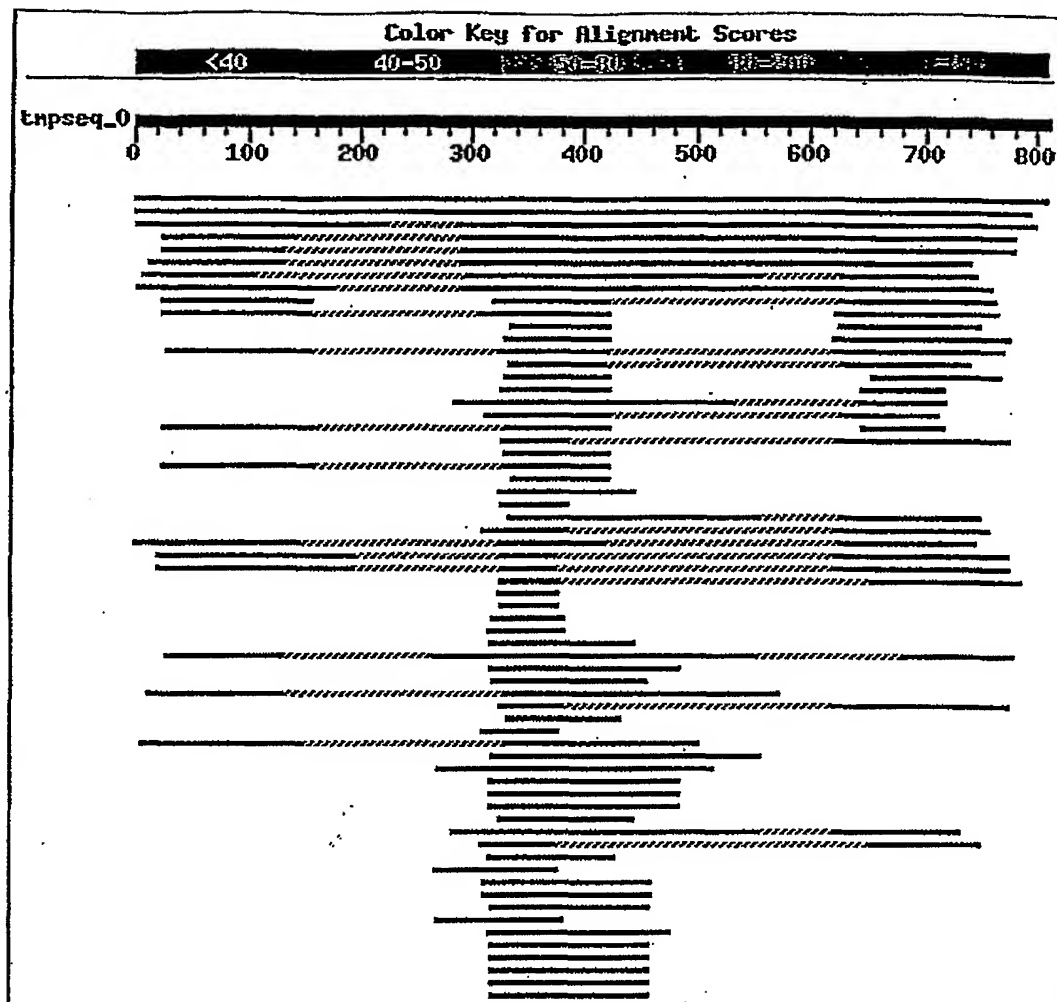
Score E
(bits) Value

| | | | |
|------------------------|--|------|-------|
| sp Q10250 YD22 SCHPO | HYPOTHETICAL 170.7 KD PROTEIN C56F8.02... | 1407 | 0.0 |
| sp Q09773 YA84 SCHPO | HYPOTHETICAL 162.4 KD PROTEIN C22F3.04... | 576 | e-163 |
| ref NP_014736.1 | Yor093cp [Saccharomyces cerevisiae] >gi 21... | 561 | e-158 |
| qb AAF64300.1 | (AF246991) unknown [Drosophila melanogaster] | 249 | 2e-64 |
| sp Q14689 Y184 HUMAN | HYPOTHETICAL PROTEIN KIAA0184 >gi 1136... | 247 | 6e-64 |
| sp Q9Y2E4 Y934 HUMAN | HYPOTHETICAL PROTEIN KIAA0934 >gi 4589... | 247 | 6e-64 |
| dbj BAA95987.F | (AB040896) KIAA1463 protein [Homo sapiens] | 212 | 3e-53 |
| pir T34061 | hypothetical protein F28B3.1 - Caenorhabditis e... | 205 | 5e-51 |
| qb AAF47364.1 | (AE003467) CG7020 gene product [Drosophila m... | 150 | 1e-34 |
| pir T34918 | polyketide synthase - Streptomyces coelicolor >... | 125 | 4e-27 |
| qb AAG02359.1 AF210249 | 18 (AF210249) peptide synthetase NRP... | 106 | 2e-21 |
| pir T18551 | saframycin Mx1 synthetase B - Myxococcus xanthu... | 106 | 2e-21 |
| sp Q10976 YT30 MYCTU | HYPOTHETICAL 67.9 KDA PROTEIN RV2930 >... | 87 | 2e-15 |
| qb AAG05812.1 AE004669 | 9 (AE004669) probable non-ribosomal ... | 86 | 3e-15 |
| pir A70635 | probable fadD31 protein - Mycobacterium tubercu... | 85 | 8e-15 |
| pir C70669 | probable acyl-CoA synthetase (EC 6.2.1.7) - Myc... | 82 | 7e-14 |
| pir F70522 | probable polyketide synthase - Mycobacterium tu... | 77 | 2e-12 |

Figure 6

| | | | |
|---------------------------|---|----|-------|
| pir B70668 | probable Acyl-CoA Synthetase - Mycobacterium tu... | 74 | 1e-11 |
| pir C70634 | probable fadD30 protein - Mycobacterium tubercu... | 73 | 2e-11 |
| qb AAB52538.1 | (U75685) acyl-CoA synthase [Mycobacterium bo...] | 73 | 2e-11 |
| emb CAB36629.1 | (AL035480) putative acyl-CoA synthase [Myco...] | 71 | 8e-11 |
| pir T31307 | type I fatty acid synthase homolog - Cryptospor... | 71 | 1e-10 |
| sp Q50586 YF21 MYCTU | HYPOTHETICAL 63.1 KDA PROTEIN RV1521 >... | 69 | 3e-10 |
| pir S73072 | u0002r protein - Mycobacterium tuberculosis >gi... | 69 | 4e-10 |
| pir B70820 | probable polyketide synthase MTCY409 - Mycobact... | 68 | 7e-10 |
| pir A70877 | probable acyl-coA synthase - Mycobacterium tuber... | 62 | 4e-08 |
| pir E70887 | probable fadD32 protein - Mycobacterium tubercu... | 60 | 3e-07 |
| pir S72716 | 4-coumarate--CoA ligase homolog - Mycobacterium... | 59 | 4e-07 |
| qb AAC83455.1 | (AF117694) malonyl CoA synthetase [Rhizobium...] | 54 | 1e-05 |
| sp P94547 LCFA BACSU | LONG-CHAIN-FATTY-ACID--COA LIGASE (LON... | 52 | 7e-05 |
| pir H72454 | probable long-chain-fatty-acid--CoA ligase APE2... | 50 | 2e-04 |
| pir T03221 | probable polyketide synthase module 1 - Strepto... | 50 | 2e-04 |
| sp P29212 LCFA ECOLI | LONG-CHAIN-FATTY-ACID--COA LIGASE (LON... | 49 | 4e-04 |
| qb AAF86393.1 AF235504_14 | (AF235504) FkbB [Streptomyces hyg...] | 48 | 6e-04 |
| sp P46450 LCFA HAEIN | LONG-CHAIN-FATTY-ACID--COA LIGASE (LON... | 48 | 6e-04 |
| sp P25464 ACVS CEPAC | DELTA-(L-ALPHA-AMINOADIPYL)-L-CYSTEINY... | 48 | 8e-04 |
| pir E69378 | long-chain-fatty-acid--CoA ligase (fadD-5) homo... | 48 | 8e-04 |
| qb AAB09715.1 | (U12891) ORF5 [Pseudomonas aeruginosa] | 48 | 0.001 |
| qb AAF00957.1 AF183408_5 | (AF183408) McyG [Microcystis aerug...] | 47 | 0.001 |
| sp O30408 TYCB BACBR | TYROCIDINE SYNTHETASE II [INCLUDES: AT... | 47 | 0.001 |
| db BAB12213.1 | (AB032549) peptide synthetase and polyketid... | 47 | 0.001 |
| qb AAF28840.1 AF118888_1 | (AF118888) malonyl CoA synthetase ... | 47 | 0.001 |
| pir A61209 | hypertension-associated protein SA - rat | 46 | 0.002 |
| qb AAF95133.1 | (AE004273) long-chain-fatty-acid--CoA ligase... | 46 | 0.003 |
| emb CAA06324.1 | (AJ005061) LchAB protein [Bacillus lichenif...] | 45 | 0.006 |
| qb AAD04758.1 | (U95370) lichenysin synthetase B; LicB [Baci...] | 45 | 0.007 |
| qb AAF41909.1 | (AE002506) long-chain-fatty-acid--CoA ligase... | 45 | 0.007 |
| emb CAB84971.1 | (AL162757) long-chain-fatty-acid--CoA-ligas... | 45 | 0.007 |
| qb AAF08797.1 AF184956_4 | (AF184956) MycC [Bacillus subtilis] | 45 | 0.010 |
| pir S19560 | proline-rich protein MP4 - mouse >gi 53182 emb ... | 44 | 0.013 |
| db BAB06823.1 | (AP001517) long-chain fatty-acid-CoA ligase... | 44 | 0.013 |
| pir T07943 | probable AMP-binding protein - rape >gi 1617272... | 44 | 0.013 |
| pir T18841 | hypothetical protein C01G6.7 - Caenorhabditis e... | 43 | 0.022 |
| qb AAF02529.1 AF150669_1 | (AF150669) long-chain-fatty-acid-C... | 43 | 0.028 |
| emb CAB38084.1 | (AJ006977) Tal [Myxococcus xanthus] | 43 | 0.028 |
| db BAA37141.1 | (AB022340) SA [Mus musculus] | 43 | 0.037 |
| pir T17428 | FK506 polyketide synthase - Streptomyces sp. (s... | 43 | 0.037 |
| db BAB10742.1 | (AB023035) 4-coumarate-CoA ligase-like prot... | 43 | 0.037 |
| pir H69545 | probable fatty-acid--CoA ligase (EC 6.2.1.-) fa... | 43 | 0.037 |
| pir T30226 | polyketide synthase - Streptomyces hygroscopicu... | 42 | 0.049 |
| pir T17463 | rifamycin polyketide synthase modules 1-3 - Amy... | 42 | 0.049 |
| ref NP_058566.1 | SA rat hypertension-associated homolog [Mu... | 42 | 0.049 |
| pir YGBSG1 | phenylalanine racemase (ATP-hydrolyzing) (EC 5.... | 42 | 0.049 |
| db BAB01855.1 | (AP000377) long-chain-fatty-acid CoA ligase... | 42 | 0.064 |
| pir T05038 | 4-coumarate--CoA ligase homolog F13C5.180 - Ara... | 42 | 0.064 |
| sp O68006 BACA-BACLI | BACITRACIN SYNTHETASE 1 (BA1) [INCLUDE... | 41 | 0.083 |
| pir H69354 | probable fatty-acid--CoA ligase (EC 6.2.1.-) fa... | 41 | 0.11 |
| pir B48013 | proline-rich proteoglycan 2 precursor, parotid ... | 41 | 0.11 |
| pir B69768 | probable acid--CoA ligase (EC 6.2.1.-) ydaB - B... | 41 | 0.11 |
| pir H71401 | probable A6 anther-specific protein - Arabidops... | 41 | 0.11 |
| qb AAF00961.1 AF183408_9 | (AF183408) McyB [Microcystis aerug...] | 41 | 0.11 |
| qb AAB92395.1 | (U97078) microcystin synthetase B [Microcyst...] | 41 | 0.11 |
| emb CAA78044.1 | (Z12000) AngR protein [Vibrio anguillarum] | 41 | 0.11 |
| pir T36248 | CDA peptide synthetase I - Streptomyces coelico... | 41 | 0.11 |
| sp P19828 ANGR VIBAN | ANGR PROTEIN >gi 68686 pir YGVCAR ang... | 41 | 0.11 |
| qb AAF26925.1 AF210843_22 | (AF210843) nonribosomal peptide s... | 41 | 0.11 |
| pir D69187 | probable acid--CoA ligase (EC 6.2.1.-) MTH657 -... | 41 | 0.11 |

| | | | | |
|------------|--------------------------------|---|-----------|------|
| <u>pir</u> | <u> T07908</u> | 4-coumarate--CoA ligase (EC 6.2.1.12) 2 - weste... | <u>41</u> | 0.11 |
| <u>pir</u> | <u> E69438</u> | probable fatty-acid--CoA ligase (EC 6.2.1.-) fa... | <u>41</u> | 0.14 |
| <u>pir</u> | <u> E69274</u> | acetyl-CoA synthetase (acs-1) homolog - Archaeo... | <u>41</u> | 0.14 |
| <u>pdb</u> | <u> 1AMU B</u> | Chain B, Phenylalanine Activating Domain Of Gram... | <u>41</u> | 0.14 |
| <u>pir</u> | <u> T28932</u> | probable 4-coumarate--CoA ligase (EC 6.2.1.12) ... | <u>41</u> | 0.14 |
| <u>sp</u> | <u> P14687 GRSA_BACBR</u> | GRAMICIDIN S SYNTHETASE I [INCLUDES: A... | <u>41</u> | 0.14 |
| <u>qb</u> | <u> AAA58718.1 </u> | (M29703) grsA-encoded protein [Brevibacillus... | <u>41</u> | 0.14 |
| <u>qb</u> | <u> AAF42473.1 AF204401_1</u> | (AF204401) actinomycin synthetase ... | <u>40</u> | 0.19 |
| <u>qb</u> | <u> AAG06687.1 AE004752_3</u> | (AE004752) long-chain-fatty-acid--... | <u>40</u> | 0.19 |
| <u>qb</u> | <u> AAG01008.1 AF288210_1</u> | (AF288210) TRK-fused protein TFG [... | <u>40</u> | 0.19 |
| <u>sp</u> | <u> P26046 ACVT_PENCH</u> | DELTA-(L-ALPHA-AMINOADIPYL)-L-CYSTEINY... | <u>40</u> | 0.19 |
| <u>sp</u> | <u> P19787 ACVS_PENCH</u> | DELTA-(L-ALPHA-AMINOADIPYL)-L-CYSTEINY... | <u>40</u> | 0.19 |
| <u>pir</u> | <u> JX0340</u> | gramicidin S synthase 2 - Bacillus brevis >gi 5... | <u>40</u> | 0.19 |
| <u>emb</u> | <u> CAB72227.1 </u> | (AL138854) putative peptide synthetase; wit... | <u>40</u> | 0.19 |
| <u>pir</u> | <u> S73073</u> | pks002a protein - Mycobacterium tuberculosis >g... | <u>40</u> | 0.25 |
| <u>dbj</u> | <u> BAA83993.1 </u> | (AB019578) mcyB [Microcystis aeruginosa] | <u>40</u> | 0.25 |
| <u>ref</u> | <u> NP_036764.1 </u> | proline-rich protein, salivary [Rattus nor... | <u>40</u> | 0.25 |
| <u>pir</u> | <u> A70669</u> | probable acyl-CoA synthetase (EC 2.3.1.-) - Myc... | <u>40</u> | 0.25 |
| <u>qb</u> | <u> AAF79612.1 AC027665_13</u> | (AC027665) F5M15.18 [Arabidopsis ... | <u>40</u> | 0.25 |
| <u>qb</u> | <u> AAF08796.1 AF184956_3</u> | (AF184956) MycB [Bacillus subtilis] | <u>40</u> | 0.25 |
| <u>pir</u> | <u> T14591</u> | actinomycin synthetase II - Streptomyces chryso... | <u>40</u> | 0.25 |
| <u>pir</u> | <u> H69371</u> | probable acid--CoA ligase (EC 6.2.1.-) AF0976 -... | <u>39</u> | 0.32 |
| <u>qb</u> | <u> AAF46366.1 </u> | (AE003443) CG10555 gene product [Drosophila ... | <u>39</u> | 0.32 |



Sequences producing significant alignments:

| | | Score | E |
|----------------------|--|--------|-------|
| | | (bits) | Value |
| sp Q10250 YD22 SCHPO | HYPOTHETICAL 170.7 KD PROTEIN C56F8.02... | 819 | 0.0 |
| ref NP_014736.1 | Yor093cp [Saccharomyces cerevisiae] >gi 21... | 277 | 3e-73 |
| sp Q09773 YA84 SCHPO | HYPOTHETICAL 162.4 KD PROTEIN C22F3.04... | 169 | 1e-40 |
| sp Q14689 Y184 HUMAN | HYPOTHETICAL PROTEIN KIAA0184 >gi 1136... | 89 | 3e-16 |
| sp Q9Y2E4 Y934 HUMAN | HYPOTHETICAL PROTEIN KIAA0934 >gi 4589... | 86 | 1e-15 |
| gb AAF64300.1 | (AF246991) unknown [Drosophila melanogaster] | 81 | 1e-14 |
| pir T18551 | saframycin Mx1 synthetase B - Myxococcus xanthu... | 80 | 1e-13 |
| pir T34061 | hypothetical protein F28B3.1 - Caenorhabditis e... | 73 | 1e-11 |
| sp Q10976 YT30 MYCTU | HYPOTHETICAL 67.9 KDA PROTEIN RV2930 >... | 72 | 2e-11 |
| pir C70669 | probable acyl-CoA synthetase (EC 6.2.1.-) - Myc... | 71 | 6e-11 |
| pir S73072 | u0002r protein - Mycobacterium tuberculosis >gi... | 68 | 3e-10 |
| pir F70522 | probable polyketide synthase - Mycobacterium tu... | 66 | 1e-09 |
| emb CAB36629.1 | (AL035480) putative acyl-CoA synthase [Myco... | 65 | 4e-09 |
| gb AAG02359.1 | AF210249 18 (AF210249) peptide synthetase NRP... | 63 | 1e-08 |
| pir B70820 | probable polyketide synthase MTCY409 - Mycobact... | 62 | 2e-08 |
| pir A70877 | probable acyl-coAsynthase - Mycobacterium tuber... | 61 | 5e-08 |
| pir A70635 | probable fadD31 protein - Mycobacterium tubercu... | 59 | 2e-07 |

Figure 7A

| | | |
|--|-----------|--------------|
| <u>pir</u> <u>T31307</u> type I fatty acid synthase homolog - Cryptospor... | <u>59</u> | <u>2e-07</u> |
| <u>sp</u> <u>Q50586</u> <u>YF21</u> MYCTU HYPOTHETICAL 63.1 KDA PROTEIN RV1521 >... | <u>59</u> | <u>2e-07</u> |
| <u>qb</u> <u>AAF95133.1</u> (AE004273) long-chain-fatty-acid--CoA ligase... | <u>59</u> | <u>2e-07</u> |
| <u>pir</u> <u>B70668</u> probable Acyl-CoA Synthetase - Mycobacterium tu... | <u>59</u> | <u>2e-07</u> |
| <u>qb</u> <u>AAB52538.1</u> (U75685) acyl-CoA synthase [Mycobacterium bo... | <u>59</u> | <u>3e-07</u> |
| <u>pir</u> <u>S72716</u> 4-coumarate--CoA ligase homolog - Mycobacterium... | <u>57</u> | <u>6e-07</u> |
| <u>pir</u> <u>E69378</u> long-chain-fatty-acid--CoA ligase (fadD-5) homo... | <u>54</u> | <u>7e-06</u> |
| <u>qb</u> <u>AAC83455.1</u> (AF117694) malonyl CoA synthetase [Rhizobium... | <u>54</u> | <u>7e-06</u> |
| <u>qb</u> <u>AAG05812.1</u> <u>AE004669</u> 2 (AE004669) probable non-ribosomal ... | <u>53</u> | <u>1e-05</u> |
| <u>emb</u> <u>CAA70871.1</u> (Y09700) rpfB [Xanthomonas campestris] | <u>52</u> | <u>2e-05</u> |
| <u>pir</u> <u>T34918</u> polyketide synthase - Streptomyces coelicolor >... | <u>52</u> | <u>4e-05</u> |
| <u>sp</u> <u>P29212</u> <u>LCFA</u> <u>ECOLI</u> LONG-CHAIN-FATTY-ACID--COA LIGASE (LON... | <u>52</u> | <u>4e-05</u> |
| <u>sp</u> <u>P46450</u> <u>LCFA</u> <u>HAEIN</u> LONG-CHAIN-FATTY-ACID--COA LIGASE (LON... | <u>52</u> | <u>4e-05</u> |
| <u>qb</u> <u>AAB09715.1</u> (U12891) ORF5 [Pseudomonas aeruginosa] | <u>50</u> | <u>1e-04</u> |
| <u>pir</u> <u>A45062</u> long-chain-fatty-acid--CoA ligase (EC 6.2.1.3) ... | <u>49</u> | <u>2e-04</u> |
| <u>qb</u> <u>AAF83100.1</u> <u>AE003882</u> 2 (AE003882) regulator of pathogenic... | <u>49</u> | <u>2e-04</u> |
| <u>pir</u> <u>H72454</u> probable long-chain-fatty-acid--CoA ligase APE2... | <u>49</u> | <u>2e-04</u> |
| <u>qb</u> <u>AAF28840.1</u> <u>AF118888</u> 1 (AF118888) malonyl CoA synthetase ... | <u>49</u> | <u>2e-04</u> |
| <u>pir</u> <u>T03221</u> probable polyketide synthase module 1 - Strepto... | <u>48</u> | <u>4e-04</u> |
| <u>qb</u> <u>AAF86393.1</u> <u>AF235504</u> 14 (AF235504) FkbB [Streptomyces hyg... | <u>48</u> | <u>5e-04</u> |
| <u>qb</u> <u>AAF79612.1</u> <u>AC027665</u> 13 (AC027665) F5M15.18 [Arabidopsis ... | <u>47</u> | <u>7e-04</u> |
| <u>pir</u> <u>S73071</u> u0002q protein - Mycobacterium tuberculosis >gi... | <u>47</u> | <u>7e-04</u> |
| <u>dbj</u> <u>BAA95987.1</u> (AB040896) KIAA1463 protein [Homo sapiens] | <u>46</u> | <u>0.001</u> |
| <u>pir</u> <u>YGBSG1</u> phenylalanine racemase (ATP-hydrolyzing) (EC 5.... | <u>46</u> | <u>0.001</u> |
| <u>pir</u> <u>T07908</u> 4-coumarate--CoA ligase (EC 6.2.1.12) 2 - weste... | <u>46</u> | <u>0.002</u> |
| <u>qb</u> <u>AAF47364.1</u> (AE003467) CG7020 gene product [Drosophila m... | <u>45</u> | <u>0.003</u> |
| <u>qb</u> <u>AAF41909.1</u> (AE002506) long-chain-fatty-acid--CoA ligase... | <u>45</u> | <u>0.003</u> |
| <u>emb</u> <u>CAB84971.1</u> (AL162757) long-chain-fatty-acid--CoA-ligas... | <u>45</u> | <u>0.003</u> |
| <u>qb</u> <u>AAD39590.1</u> <u>AC007858</u> 4 (AC007858) This gene is a member o... | <u>45</u> | <u>0.005</u> |
| <u>dbj</u> <u>BAB06823.1</u> (AP001517) long-chain fatty-acid-CoA ligase... | <u>44</u> | <u>0.006</u> |
| <u>pir</u> <u>C70634</u> probable fadD30 protein - Mycobacterium tubercu... | <u>44</u> | <u>0.008</u> |
| <u>qb</u> <u>AAF08795.1</u> <u>AF184956</u> 2 (AF184956) MycA [Bacillus subtilis] | <u>44</u> | <u>0.008</u> |
| <u>pir</u> <u>E69438</u> probable fatty-acid--CoA ligase (EC 6.2.1.-) fa... | <u>43</u> | <u>0.010</u> |
| <u>pdb</u> <u>1AMU</u> <u>B</u> Chain B, Phenylalanine Activating Domain Of Gram... | <u>43</u> | <u>0.013</u> |
| <u>pir</u> <u>T18841</u> hypothetical protein C01G6.7 - Caenorhabditis e... | <u>43</u> | <u>0.013</u> |
| <u>sp</u> <u>P14687</u> <u>GRSA</u> <u>BACBR</u> GRAMICIDIN S SYNTHETASE I [INCLUDES: A... | <u>43</u> | <u>0.013</u> |
| <u>qb</u> <u>AAA58718.1</u> (M29703) grsA-encoded protein [Brevibacillus... | <u>43</u> | <u>0.013</u> |
| <u>sp</u> <u>P94547</u> <u>LCFA</u> <u>BACSU</u> LONG-CHAIN-FATTY-ACID--COA LIGASE (LON... | <u>42</u> | <u>0.023</u> |
| <u>pir</u> <u>E70887</u> probable fadD32 protein - Mycobacterium tubercu... | <u>42</u> | <u>0.023</u> |
| <u>qb</u> <u>AAF79611.1</u> <u>AC027665</u> 12 (AC027665) F5M15.17 [Arabidopsis ... | <u>42</u> | <u>0.030</u> |
| <u>pir</u> <u>T17463</u> rifamycin polyketide synthase modules 1-3 - Amy... | <u>42</u> | <u>0.030</u> |
| <u>pir</u> <u>C69471</u> probable fatty-acid--CoA ligase (EC 6.2.1.-) fa... | <u>42</u> | <u>0.030</u> |
| <u>sp</u> <u>Q01886</u> <u>HTS1</u> <u>COCCA</u> HC-TOXIN SYNTHETASE (HTS) >gi 167219 g... | <u>41</u> | <u>0.039</u> |
| <u>pir</u> <u>A45086</u> HC-toxin synthetase - fungus (Cochliobolus carb... | <u>41</u> | <u>0.039</u> |
| <u>pir</u> <u>T07943</u> probable AMP-binding protein - rape >gi 1617272... | <u>41</u> | <u>0.039</u> |
| <u>qb</u> <u>AAD40664.1</u> <u>AF150686</u> 1 (AF150686) 4-coumarate:coenzyme A ... | <u>41</u> | <u>0.039</u> |
| <u>sp</u> <u>P31685</u> <u>4CL2</u> <u>SOLTU</u> 4-COUMARATE--COA LIGASE 2 (4CL) >gi 10... | <u>41</u> | <u>0.051</u> |
| <u>pir</u> <u>T30226</u> polyketide synthase - Streptomyces hygroscopicu... | <u>41</u> | <u>0.051</u> |
| <u>sp</u> <u>P14912</u> <u>4CL1</u> <u>PETCR</u> 4-COUMARATE--COA LIGASE 1 (4CL) >gi 82... | <u>41</u> | <u>0.051</u> |
| <u>qb</u> <u>AAD34542.1</u> <u>AF139644</u> 1 (AF139644) luciferase [Phrixothrix... | <u>41</u> | <u>0.051</u> |
| <u>pir</u> <u>T28932</u> probable 4-coumarate--CoA ligase (EC 6.2.1.12) ... | <u>41</u> | <u>0.051</u> |
| <u>sp</u> <u>P31684</u> <u>4CL1</u> <u>SOLTU</u> 4-COUMARATE--COA LIGASE 1 (4CL) >gi 10... | <u>41</u> | <u>0.051</u> |
| <u>pir</u> <u>T09755</u> 4-coumarate--CoA ligase (EC 6.2.1.12) 4CL2 - lo... | <u>41</u> | <u>0.051</u> |
| <u>pir</u> <u>T05038</u> 4-coumarate--CoA ligase homolog F13C5.180 - Ara... | <u>41</u> | <u>0.051</u> |
| <u>sp</u> <u>P14913</u> <u>4CL2</u> <u>PETCR</u> 4-COUMARATE--COA LIGASE 2 (4CL) >gi 28... | <u>41</u> | <u>0.051</u> |
| <u>qb</u> <u>AAF37732.1</u> <u>AF052221</u> 1 (AF052221) 4-coumarate--CoA ligase... | <u>41</u> | <u>0.067</u> |
| <u>qb</u> <u>AAF37733.1</u> <u>AF052222</u> 1 (AF052222) 4-coumarate--CoA ligase... | <u>41</u> | <u>0.067</u> |
| <u>pir</u> <u>T03390</u> 4-coumarate--CoA ligase' (EC 6.2.1.12) isoform 2... | <u>41</u> | <u>0.067</u> |
| <u>pir</u> <u>T17428</u> FK506 polyketide synthase - Streptomyces sp. (s... | <u>41</u> | <u>0.067</u> |
| <u>emb</u> <u>CAB81058.1</u> (AL161502) 4-coumarate--CoA ligase-like pro... | <u>41</u> | <u>0.067</u> |

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|------------|---------------------------|--|-----------|--------------|
| <u>pir</u> | <u> T07909</u> | 4-coumarate--CoA ligase (EC 6.2.1.12) 1 - weste... | <u>40</u> | <u>0.088</u> |
| <u>gb</u> | <u> AAB18637.1</u> | (U50845) 4-coumarate:coenzyme A ligase [Nico... | <u>40</u> | <u>0.088</u> |
| <u>gb</u> | <u> AAF02529.1</u> | <u> AF150669 1</u> (AF150669) long-chain-fatty-acid-C... | <u>40</u> | <u>0.088</u> |
| <u>gb</u> | <u> AAF91310.1</u> | <u> AF239687 1</u> (AF239687) 4-coumarate:CoA ligase ... | <u>40</u> | <u>0.088</u> |
| <u>gb</u> | <u> AAG29784.1</u> | <u> AF235050 7</u> (AF235050) putative ligase [Strept... | <u>40</u> | <u>0.12</u> |
| <u>gb</u> | <u> AAG06688.1</u> | <u> AE004752 4</u> (AE004752) long-chain-fatty-acid-... | <u>40</u> | <u>0.12</u> |
| <u>pir</u> | <u> C75364</u> | long-chain fatty acid--CoA ligase - Deinococcus... | <u>39</u> | <u>0.15</u> |
| <u>pir</u> | <u> H69354</u> | probable fatty-acid--CoA ligase (EC 6.2.1.-) fa... | <u>39</u> | <u>0.15</u> |
| <u>sp</u> | <u> O30408 TYCB_BACBR</u> | TYROCIDINE SYNTHETASE II [INCLUDES: AT... | <u>39</u> | <u>0.15</u> |
| <u>pir</u> | <u> H69545</u> | probable fatty-acid--CoA ligase (EC 6.2.1.-) fa... | <u>39</u> | <u>0.15</u> |
| <u>dbj</u> | <u> BAA05006.1</u> | (D25416) luciferase [Photuris pennsylvanica] | <u>39</u> | <u>0.20</u> |
| <u>pir</u> | <u> A70551</u> | probable acid--CoA ligase (EC 6.2.1.-) fadD35 -... | <u>39</u> | <u>0.20</u> |
| <u>pir</u> | <u> S73073</u> | pks002a protein - Mycobacterium tuberculosis >g... | <u>39</u> | <u>0.26</u> |
| <u>sp</u> | <u> P17814 4CL_ORYSA</u> | 4-COUMARATE--COA LIGASE >gi 82454 pir ... | <u>39</u> | <u>0.26</u> |
| <u>emb</u> | <u> CAB84715.1</u> | (AL162756) putative acyl-CoA ligase [Neisse... | <u>39</u> | <u>0.26</u> |
| <u>pir</u> | <u> H69371</u> | probable acid--CoA ligase (EC 6.2.1.-) AF0976 -... | <u>39</u> | <u>0.26</u> |
| <u>pir</u> | <u> A70669</u> | probable acyl-CoA synthetase (EC 2.3.1.-) - Myc... | <u>39</u> | <u>0.26</u> |
| <u>emb</u> | <u> CAA53230.1</u> | (X75542) 4-coumarate:CoA ligase [Vanilla pl... | <u>39</u> | <u>0.26</u> |
| <u>dbj</u> | <u> BAA05005.1</u> | (D25415) luciferase [Photuris pennsylvanica] | <u>39</u> | <u>0.26</u> |
| <u>gb</u> | <u> AAF41652.1</u> | (AE002476) long-chain-fatty-acid--CoA ligase... | <u>39</u> | <u>0.26</u> |
| <u>dbj</u> | <u> BAB01715.1</u> | (AB023045) 4-coumarate:CoA ligase [Arabidop... | <u>39</u> | <u>0.26</u> |
| <u>pir</u> | <u> T36202</u> | probable fatty acid--CoA ligase - Streptomyces ... | <u>38</u> | <u>0.34</u> |
| <u>pir</u> | <u> H71401</u> | probable A6 anther-specific protein - Arabidops... | <u>38</u> | <u>0.34</u> |

Alignment Report of Untitled, using Clustal method with PAM250 residue weight table.

| | | Majority | | | | | | | | | | |
|-----|--|----------------------------|-----|-----|-----|-----|-----|-----|--|--|--|--|
| | | 10 | 20 | 30 | 40 | 50 | 60 | 70 | | | | |
| 1 | MLEVN | -----QGYFSDFTGQQMQDNRD | | | | | | | | | | |
| 1 | | -----AsolaniCPS1pro.PRO | | | | | | | | | | |
| 1 | | -----pteresCPS1pro.PRO | | | | | | | | | | |
| 1 | MMSGDYAFRPEQQGTYESQ | -----FGcps1pro.PRO | | | | | | | | | | |
| 1 | | -----C.immitis CPS1pro.PRO | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | Majority | | | | | | | | | | |
| | | 80 | 90 | 100 | 110 | 120 | 130 | 140 | | | | |
| 23 | S--YGGPN-RYSSGDAFSPTAA | -----cps1pro.PRO | | | | | | | | | | |
| 1 | | -----AsolaniCPS1pro.PRO | | | | | | | | | | |
| 1 | | -----pteresCPS1pro.PRO | | | | | | | | | | |
| 71 | TVEYVGPOQRYSSDAFSPTAAMAPMLTTNDLPPPEALEYQLPLDPREVPFAIQDPHDDSTPMSKFDNI | -----C.immitis CPS1pro.PRO | | | | | | | | | | |
| 1 | | -----C.immitis CPS1pro.PRO | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | Majority | | | | | | | | | | |
| | | 150 | 160 | 170 | 180 | 190 | 200 | 210 | | | | |
| 89 | GAVLRHRSRTQPRTTAFWVLD | -----cps1pro.PRO | | | | | | | | | | |
| 1 | | -----AsolaniCPS1pro.PRO | | | | | | | | | | |
| 1 | | -----pteresCPS1pro.PRO | | | | | | | | | | |
| 141 | AAVLRHGRGRTIAKKPAYWVLD | -----FGcps1pro.PRO | | | | | | | | | | |
| 1 | | -----C.immitis CPS1pro.PRO | | | | | | | | | | |

Figure 7B

Alignment Report of Untitled, using Clustal method with PAM250 residue weight table.

| | 220 | 230 | 240 | 250 | 260 | 270 | 280 | |
|------|---------------------------------|-----------------------------|-------------|---------------|----------------|---------|------------|-----------------------|
| 1159 | VVALMGC | FIAGVAVPINSVDDYQKILILLTTQAH | LALTTQAH | LALTTDNNLKA | FHRDISQ | NRLKWP | SGVEMWK | TNE cpslpro.PRO |
| 1 | | | | | | | | AsolaniCPSlpro.PRO |
| 1 | | | | | | | | pteresCPSlpro.PRO |
| 211 | AIALGCFIAGVAVPINDYQORLNHILTTQAH | LALTTQAH | LALTTDNNLKA | FORDITTQKLT | TPKSGVEMWK | TNE | | Fgcpslpro.PRO |
| 1 | | | | | | | | C.immitis CPSlpro.PRO |
| 229 | FGSYHPKKDD-PAL-VPDLAYIEFSR | APTGD | LRGVLSHRT | IMHQM | AC-SAI | ISTIPTN | | Majority |
| 229 | EGSEHPKKDDTPALQVPEVAYIEFSR | APTGD | LRGVLSHRT | IMHQM | ACISAMISTIPTN | AQS | DTFST | cpslpro.PRO |
| 1 | | | | | | | | AsolaniCPSlpro.PRO |
| 1 | | | | | | | | pteresCPSlpro.PRO |
| 281 | EGSYHPKKDDVPALVPDLAYIEFSR | APTGD | LRGVLSHRT | IMHQM | AC-SAI | ISTIPTN | GGP-DTENPS | Fgcpslpro.PRO |
| 110 | FGSYHPKKDEMPPLAVPDLAYIEFSR | APTGD | LRGVLSHRT | IMHQM | COMSAIVSTIPTDS | | | C.immitis CPSlpro.PRO |
| 299 | --D-GK-V--PS--EIL-SYLD | PROGIGMILSVLLTVYGGHTT | VMLE--AVET | PGLYAHLIT | TKYS | | | Majority |
| 299 | LRDAEGKFVAPAPSRNPTEVILTYLD | PPRESACILSVLF | AVYGGHTT | VWLETATMET | PGLYAHLIT | TKYS | | cpslpro.PRO |
| 1 | | | | | | | | AsolaniCPSlpro.PRO |
| 1 | | | | | | | | pteresCPSlpro.PRO |
| 350 | LRDKNCRLLGGAS--SELLVSYLD | PROGIGMILSVLLTVYGGHTT | VFDNKAVD | VPGLYAHLIT | TKYS | | | Fgcpslpro.PRO |
| 71 | --NNSGKPVPR-PH--GELLMSYLD | PROGIGMILSVLLTVYAGNTT | VWLES | AVETPGLYASLIT | TKYRA | | | C.immitis CPSlpro.PRO |

Alignment Report of Unfiled, using Clustal method with PAM250 residue weight table.

| -LL-ADYPGLKRAAYNYQQDPMATRNFKN-ERNF-S-KLCLIDTLITVDCEHFEILLADRWLRPLRNPRAR Majority | | | | | | | | | | |
|--|---|-----|-----|-----|-----|-----|-----|--|--|--|
| | 430 | 440 | 450 | 460 | 470 | 480 | 490 | | | |
| 369 | NILLADYPGLKRAAYNYQQDPMATRNFKNTEPNFASVKI CLIDTLITVDCEHFEILLGDRYFRPLRNPRAR | | | | | | | | | |
| 1 | ----- | | | | | | | | | |
| 1 | ----- | | | | | | | | | |
| 417 | TIMTADYPGLKRAAYNYQQDPMATRNFKNTEPNFQOMI KLCLIDTLITVDCEHFEILLADRWLRPLRNPRAR | | | | | | | | | |
| 135 | ALLAADYPGLKRAAYNYQQDPMATRNFKNSEPNFSSIL KLCLIDTLITVDCEHFEILLADRWLRPLRNPRAR | | | | | | | | | |
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DPETGLLCSFYSIGEIWVDSPLSGGFWQLQKHTETIFHARPYRFEVGSPTQLLEFLRTLGLGFVE Majority

CORE 3
CORE 4

GKIFVLGLYEDRIRQORVEWVEHGQLEAEHRYFFVQHLVTSIMKAVPKIYDCSSFDSYVNGEYLPILLIET Majority

507E

QAASTAPTNPGGPPQQLDIPFLDSLSERCMEVLYQEHHLRVCVMITAPNTLPRVVKNGRREIGNMLCRR Majority

COFF 6

| | | |
|-----|--|-----------------------|
| 710 | QAASTAPTNPGGPPQQLDIPFLDLSERCMEVLYQEHHLRVVYVMITAPNTLPRVTLKNGRREIGNMLCRR | cps1pro.pro |
| 200 | QAASTAPTNPGGPPQQLDIPFLDLSERCMEVLYQEHHLRVVYVMITAPNTLPRVTLKNGRREIGNMLCRR | AsolaniCPS1pro.pro |
| 200 | QAASTAPTNPGGPPQQLDIPFLDLSERCMEVLYQEHHLRVVYVMITAPNTLPRVTVKNGRREIGNMLCRR | pteresCPS1pro.pro |
| 758 | AAASTAPLTSGGPPRQPD TALL ESIAERCMEVLMS EHHLRL YCVVMITAPD TL PRVVKNGRREIGNMLCRR | Fgcps1pro.pro |
| 482 | YTA STAP VAS Q SPRQ LD V FL DDSLAEKCM CV LYQEHHLRVVYVMITAPNTLPRVTLKNGR OE IGNMLCRK | C.immitis CPS1pro.pro |

Alignment Report of Untitled. usha Clustal method with PAM250 residue weight table.

| | 850 | 860 | 870 | 880 | 890 | 900 | 910 | Majority |
|-----|--|------|-----|-------|-------|-----|-------|-----------------------------|
| | EFDNGSLPCVHVKFG | TERS | QNI | ALGDD | PAGCM | WSE | ASWAR | QQLMLQDKQYSGVDHREVVIDDRTSTP |
| 780 | EFDNGSLPCVHVKFG | TERS | QNI | ALGDD | PAGCM | WSE | ASWAR | QQLMLQDKQYSGVDHREVVIDDRTSTP |
| 270 | EFDNGSLPCVHVKFG | TERS | QNI | ALGDD | PAGCM | WSE | ASWAR | QQLMLQDKQYSGVDHREVVIDDRTSTP |
| 270 | EFDNGSLPCVHVKFG | TERS | QNI | ALGDD | PAGCM | WSE | ASWAR | QQLMLQDKQYSGVDHREVVIDDRTSTP |
| 828 | EFDNGSLPCVHVKFG | TERS | QNI | ALGDD | PAGCM | WSE | ASWAR | QQLMLQDKQYSGVDHREVVIDDRTSTP |
| 552 | EFDNGSLPCVHVKFG | TERS | QNI | ALGDD | PAGCM | WSE | ASWAR | QQLMLQDKQYSGVDHREVVIDDRTSTP |
| | LNQFSNIHDLQMRVSRQAEELAYCTVDGRGKEGKGVNWKFFDQKVAGVAMYLKKNKVKVQTGDHLLMYT | | | | | | | Majority |
| 850 | LNQFSNIHDLQMRVSRQAEELAYCTVDGRGKEGKGVNWKFFDQKVAGVAMYLKKNKVKVQTGDHLLMYT | | | | | | | cpslpro.PRO |
| 340 | LNQFSNIHDLQMRVSRQAEELAYCTVDGRGKEGKGVNWKFFDQKVAGVAMYLKKNKVKVQTGDHLLMYT | | | | | | | AsolaniCPSlpro.PRO |
| 340 | LNQFSNIHDLQMRVSRQAEELAYCTVDGRGKEGKGVNWKFFDQKVAGVAMYLKKNKVKVQTGDHLLMYT | | | | | | | pteresCPSlpro.PRO |
| 898 | LNQFSNIHDLQMRVSRQAEELAYCTVDGRGKEGKGVNWKFFDQKVAGVAMYLKKNKVKVQTGDHLLMYT | | | | | | | Fgcpslpro.PRO |
| 622 | LNQFSNIHDLQMRVSRQAEELAYCTVDGRGKEGKGVNWKFFDQKVAGVAMYLKKNKVKVQTGDHLLMYT | | | | | | | C.immitis CPSlpro.PRO |
| | HSEEFVYAVHACFVLGAVCIPMAPIDQNRINEDAPALLHILADEFKVKAILVNADVVDHLMKVKQVSQHIKQ | | | | | | | Majority |
| 920 | HSEEFVYAVHACFVLGAVCIPMAPIDQNRINEDAPALLHILADEFKVKAILVNADVVDHLMKVKQVSQHIKQ | | | | | | | cpslpro.PRO |
| 410 | HSEEFVYAVHACFVLGAVCIPMAPIDQNRINEDAPALLHILADEFKVKAILVNADVVDHLMKVKQVSQHIKQ | | | | | | | AsolaniCPSlpro.PRO |
| 410 | HSEEFVYAVHACFVLGAVCIPMAPIDQNRINEDAPALLHILADEFKVKAILVNADVVDHLMKVKQVSQHIKQ | | | | | | | pteresCPSlpro.PRO |
| 968 | HSEEFVYAVHACFVLGAVCIPMAPIDQNRINEDAPALLHILADEFKVKAILVNADVVDHLMKVKQVSQHIKQ | | | | | | | Fgcpslpro.PRO |
| 692 | HSEEFVYAVHACFVLGAVCIPMAPIDQNRINEDAPALLHILADEFKVKAILVNADVVDHLMKVKQVSQHIKQ | | | | | | | C.immitis CPSlpro.PRO |

Col 1

Alignment Report of Untitled, using Clustal method with PAM250 residue weight table.

| | | |
|------|--|-----------------------|
| | SAAILKISVPNTYNTTKPPKQSSGCRDLKLTIRPAWIOGFFVLVWYTWTPDQRRIAVQLGHSQIMALCK | Majority |
| | 1060 1070 1080 1090 1100 1110 1120 | |
| 990 | SAAILKISVPNTYNTTKPPKQSSGCRDLKLTIRPAWIOGFFVLVWYTWTPDQRRIAVQLGHSQIMALCK | cpslpro.PRO |
| 480 | SAAILKINVPNTYNTTKPPKQSSGCRDLKLTIRPAWIOGFFVLVWYTWTPDQRRIAVQLGHSQIMALCK | AsolaniCPSlpro.PRO |
| 480 | SAALFKINVPNTYNTTKPPKQSSGCRDLKLTIRPAWIOGFFVLVWYTWTPDQRRIAVQLGHSQIMALCK | pteresCPSlpro.PRO |
| 1038 | SAQVLKITSPLIYNTTKPPKQSSGCRDLKLTIRPAWIOGFFVLVWYTWTPDQRRISVQLGHDITIMGMCK | Fgcpslpro.PRO |
| 762 | SAHVVRTSVPSVNTSKPPKQSSGCRDLKLTIRPAWIOGFFVLVWYTWTPDQRRISVQLGHDITIMGMCK | C.immitis CPSlpro.PRO |
| | VQKETCQMTSTRPVLGCVRSTIGLGFHTCLMGIFLAAPTIVSPVDFEAQNPNIILFQTLTRYKIKDAYAT | Majority |
| | 1130 1140 1150 1160 1170 1180 1190 | |
| 1060 | VQKETCQMTSTRPVLGCVRSTIGLGFHTCLMGIFLAAPTIVSPVDFEAQNPNIILFQTLTRYKIKDAYAT | cpslpro.PRO |
| 550 | VQKETCQMTSTRPVLGCVRSTIGLGFHTCLMGIFLAAPTIVSPVDFEAQNPNIILFQTLTRYKIKDAYAT | AsolaniCPSlpro.PRO |
| 550 | VQKETCQMTSTRPVLGCVRSTIGLGFHTCLMGIFLAAPTIVSPVDFEAQNPNIILFQTLTRYKIKDAYAT | pteresCPSlpro.PRO |
| 1108 | VQKETCQMTSTRPVLGCVRSTIGLGFHTCLMGIFLAAPTIVSPVDFEAQNPNIILFQTLTRYKIKDAYAT | Fgcpslpro.PRO |
| 811 | VQKETCQMTSTRPVLGCVRSTIGLGFHTCLMGIFLAAPTIVSPVDFEAQNPNIILFQTLTRYKIKDAYAT | C.immitis CPSlpro.PRO |
| | SQMLDHAIAARGAGKNMALHELKKNLMIAATDGRPRVDVYQVRVHFAPASLDRTAINTVYSHVLNPMVASRS | Majority |
| | 1200 1210 1220 1230 1240 1250 1260 | |
| 1130 | SQMLDHAIAARGAGKNMALHELKKNLMIAATDGRPRVDVYQVRVHFAPASLDRTAINTVYSHVLNPMVASRS | cpslpro.PRO |
| 620 | SQMLDHAIAARGAGKNMALHELKKNLMIAATDGRPRVDVYQVRVHFAPASLDRTAINTVYSHVLNPMVASRS | AsolaniCPSlpro.PRO |
| 620 | SQMLDHAIAARGAGKNMALHELKKNLMIAATDGRPRVDVYQVRVHFAPASLDRTAINTVYSHVLNPMVASRS | pteresCPSlpro.PRO |
| 1178 | PQMLDHAMNSMQAKGFTLHELKKNMIIAASRPRVDVYQVRVHFAPASLDRTAINTVYSHVLNPMVASRS | Fgcpslpro.PRO |
| 811 | PQMLDHAMNSMQAKGFTLHELKKNMIIAASRPRVDVYQVRVHFAPASLDRTAINTVYSHVLNPMVASRS | C.immitis CPSlpro.PRO |

Alignment Report of Untitled, using Clustal method with PAM250 residue weight table.

| YMCIEPIELHLDVXALRRGLVMPVDPDTEPGALLVQDSGMVPVSTQIAIVNPETNQLCLNGEYGEIIV-S Majority | | | | | | | | | |
|---|---|------|------|------|------|------|------|-----------------------|--|
| | 1270 | 1280 | 1290 | 1300 | 1310 | 1320 | 1330 | | |
| 1200 | YMCIEPIELHLDVXALRRGLVMPVDPDTEPNALLVQDSGMVPVSTQISIVNPETNQLCLNGEYGEIIVQS | | | | | | | cpslpro.PRO | |
| 690 | YMCIEPIELHLDVXALRRGLVMPVDPDTEPGALLVQDSGMVPVSTQISIVNPETNQLCLNGEYGEIIV-- | | | | | | | AsolaniCPSlpro.PRO | |
| 690 | YMCIEPIELHLDVXALRRGLVMPVDPDTEPGALLVQDSGMVPVSTQIAIVNPETNQLCLNGEYGEIIV-- | | | | | | | pteresCPSlpro.PRO | |
| 1248 | YMCIEPIELHLDVXALRRGLVMPVDPDTEPGALLVQDSGMVPVSTQIAIVNPESRIHCLDGEYGEIIVQDS | | | | | | | Fgcpslpro.PRO | |
| 811 | | | | | | | | C.immitis CPSlpro.PRO | |
| EA---SFY-SK---DAERF-GR--DGDPN--Y-RTGDLGFLH-V-RPIGPNGA-VDMQVLFVLG-IG-TF Majority | | | | | | | | | |
| | 1340 | 1350 | 1360 | 1370 | 1380 | 1390 | 1400 | | |
| 1270 | EANAYSFYMSKERLDAERFNGRTIDGDPNVRYVRTGDLGFLHVSRRPIGPNGAPVDMQVLFVLGSGIDTF | | | | | | | cpslpro.PRO | |
| 758 | | | | | | | | AsolaniCPSlpro.PRO | |
| 758 | | | | | | | | pteresCPSlpro.PRO | |
| 1318 | EACVKSFYGSKDAERFDGRALDGDPNICVIRTGDLGFLHVSRRPIGPNGAQVDMQVLFVLGSGIDTF | | | | | | | Fgcpslpro.PRO | |
| 811 | | | | | | | | C.immitis CPSlpro.PRO | |
| E-NGL-HF-MDIE-SVE-CHRNIV--GCAVFQAGGLVVV-VE--R--LAS-VPVIVNAILNEHQ---DI Majority | | | | | | | | | |
| | 1410 | 1420 | 1430 | 1440 | 1450 | 1460 | 1470 | | |
| 1340 | EVNGLNHFSDIEQSVRCHRNIVPGGCAVFQAGGLVVVVEI FRNF LASMPVIVNAILNEHQIIVDI | | | | | | | cpslpro.PRO | |
| 758 | | | | | | | | AsolaniCPSlpro.PRO | |
| 758 | | | | | | | | pteresCPSlpro.PRO | |
| 1388 | EINGLSHFPMDIENSVEKCHRNIVANGCAVFQAGGLVVVVEVNRKPYLASIPVIVNAILNEHQIIVDI | | | | | | | Fgcpslpro.PRO | |
| 811 | | | | | | | | C.immitis CPSlpro.PRO | |

Alignment Report of Unfiled, using Clustal method with PAM250 residue weight table.

| V-FV-KGDF-RSRLGEKQKIL-GWV-RK-RT-AQ-SIRD-----P--RASM-----P Majority | | | | | | | | | |
|--|------------------------------|---|------------------|-----------------|---------------|----------|--------------|--|--|
| | 1480 | 1490 | 1500 | 1510 | 1520 | 1530 | 1540 | | |
| 1410 | VSVQKGF | ERSRLGEKQKILAGWVTRKMTTAQYSIRDENCQDSQMITTEEPGPRASMTGSMGLGRMG | | | | | | | |
| 758 | | | | | | | | | |
| 758 | | | | | | | | | |
| 1458 | VAFNKGDF | ERSRLGEKQKILGGWVSRKLRRTLAQFSIRDMDAESTAGDMDEPS-RASMV | | | | | | | |
| 811 | | | | | | | | | |
| -----S-----G-----AP-----N-----Q-----M-P-----P-----EL Majority | | | | | | | | | |
| | 1550 | 1560 | 1570 | 1580 | 1590 | 1600 | 1610 | | |
| 1480 | PASIKACSTRAPSLMGMTATMNNLSLTQ | QQQQQQYQPGMYAQQQQWHFQQQHQFMSNTFPQGPQGV | | | | | | | |
| 758 | | | | | | | | | |
| 758 | | | | | | | | | |
| 1518 | --SVRS | GGGAAG | -----SSSLRN | VEPAPQILEE | -----EHDQMT | PRHEYE | -----AAETWIS | | |
| 811 | | | | | | | | | |
| -----D-----TPT-----HS-----P-----G-----P-----P-----Q-----P Majority | | | | | | | | | |
| | 1620 | 1630 | 1640 | 1650 | 1660 | 1670 | 1680 | | |
| 1550 | HDPSDR | TPTDNRHSFLAD | PRMQNQGMNHTGAYEP | MNYQAYHPEHQOYSE | DGSRLSCEVPDVL | LRP | | | |
| 758 | | | | | | | | | |
| 758 | | | | | | | | | |
| 1566 | PD-Q | QETPTG | QHSQYEHPP | -----QSAC | SOAP | QALNLSHQ | PDG | | |
| 811 | | | | | | | | | |

Sequencing strategy

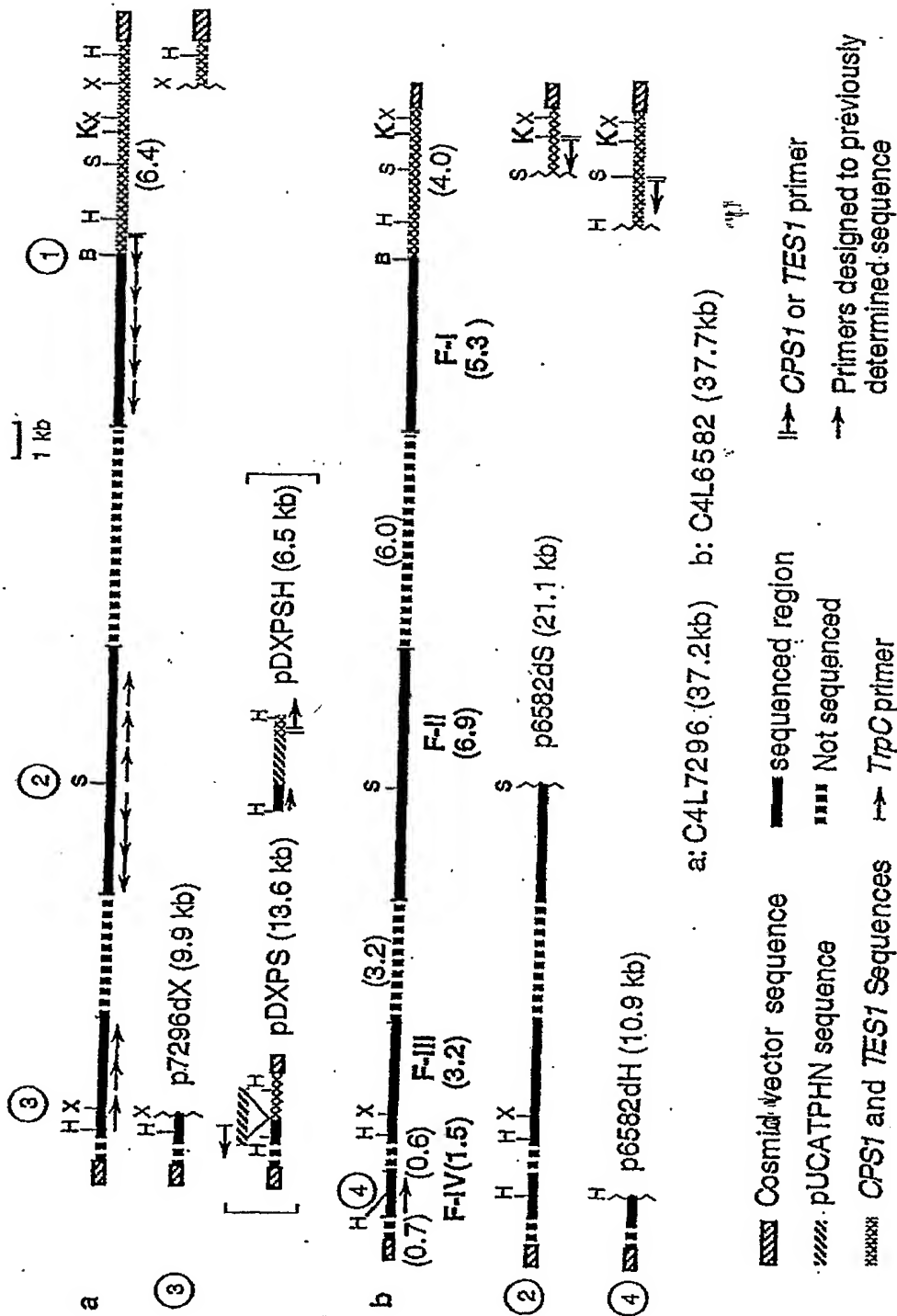


Figure 8A

Restriction maps of sequenced fragments.

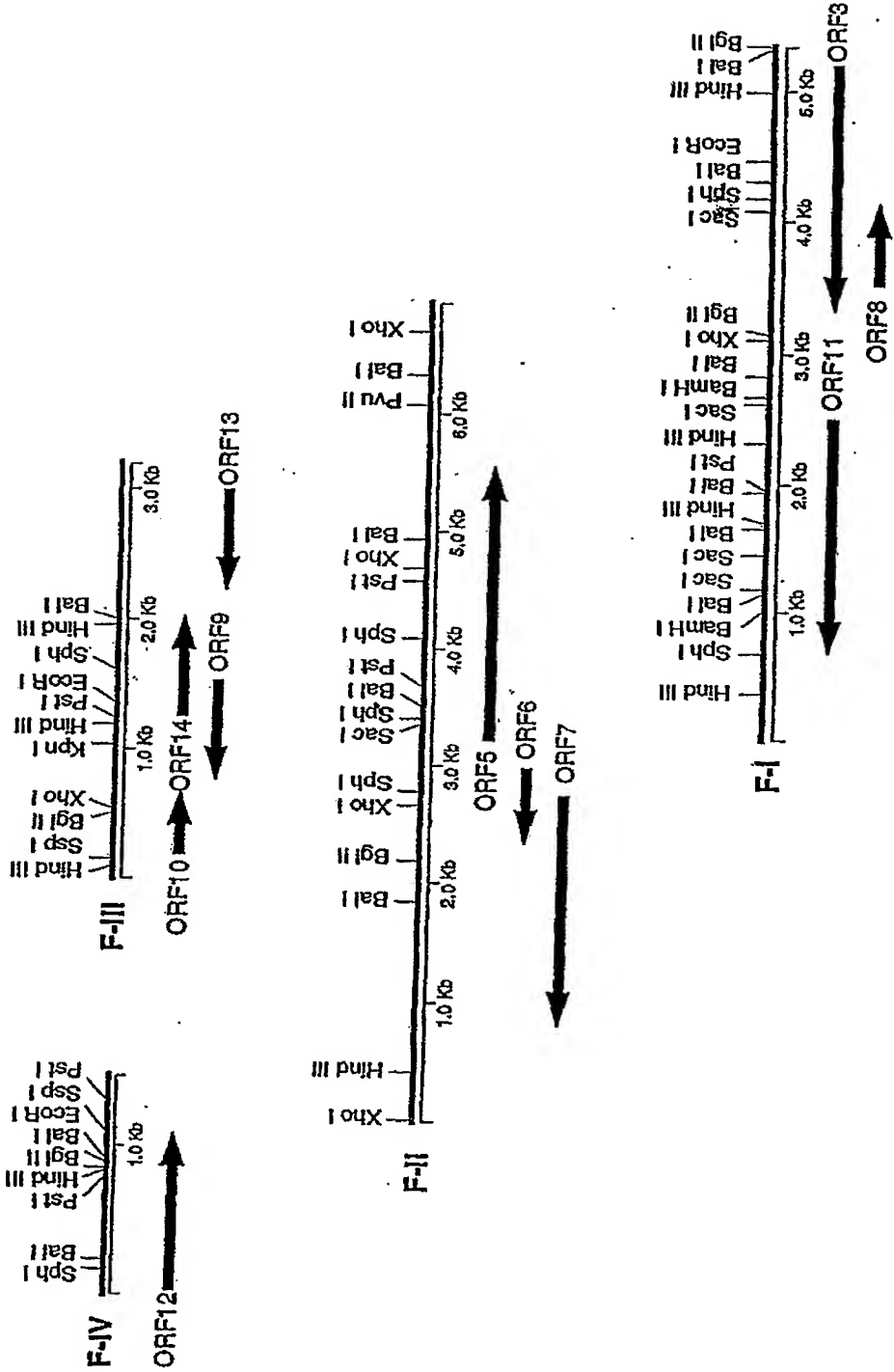
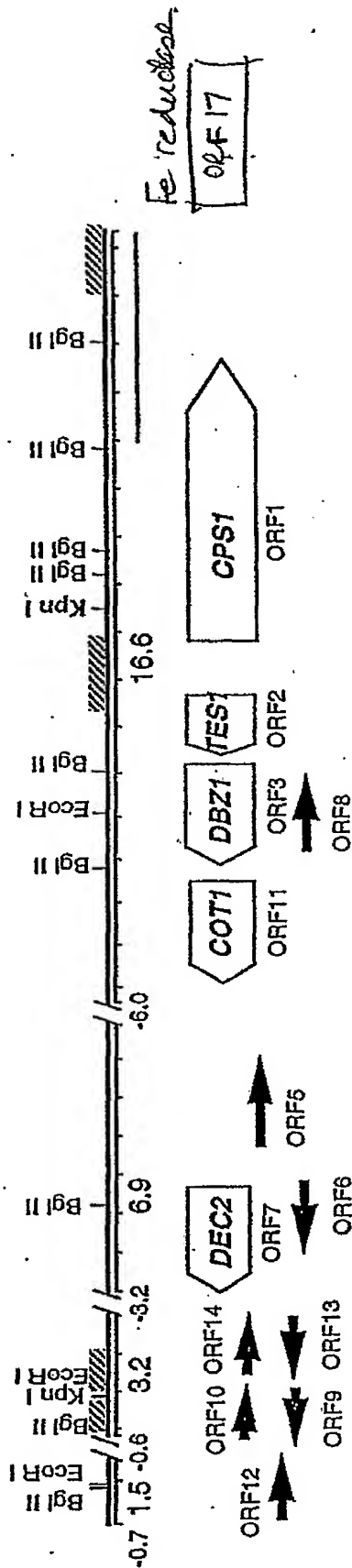
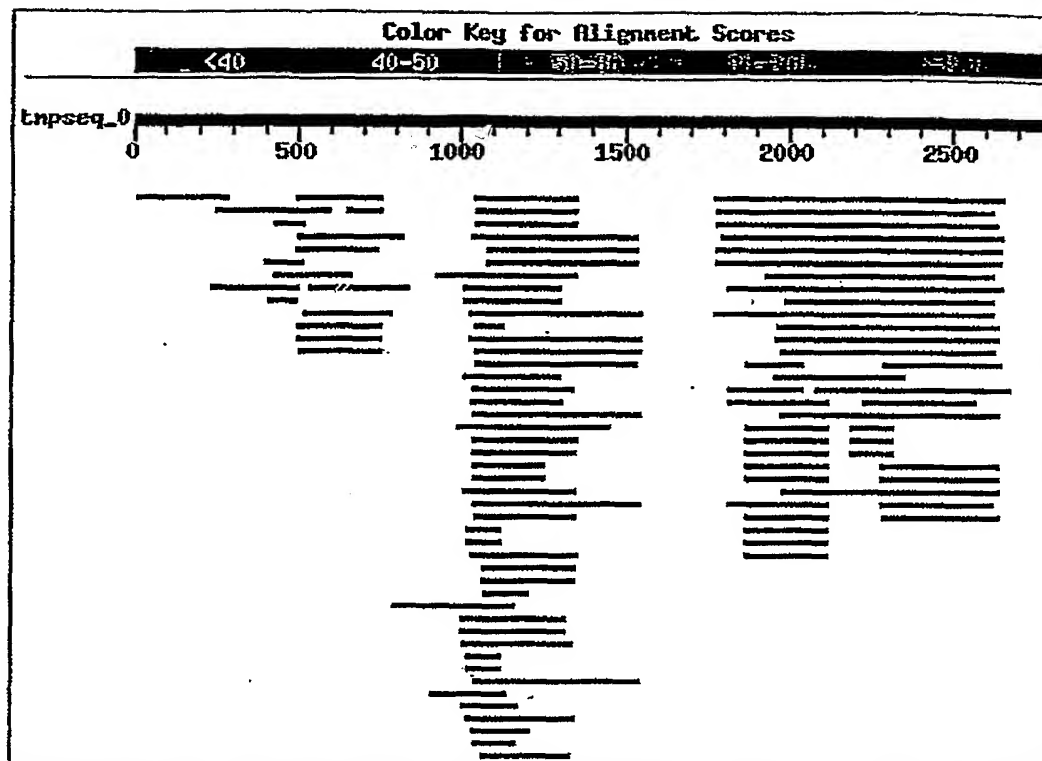


Figure 8B



QEP-15 permease, MFS transporter
QEP-16 lactase precursor

Figure 8c



Sequences producing significant alignments:

| | Score | E |
|--|--------|-------|
| | (bits) | Value |
| ref NP_015026.1 similar to FRE2; Fre3p [Saccharomyces cere... | 106 | |
| ref NP_012702.1 [Saccharomyces cer... | 106 | |
| gb AAG09788.1 AF254143.1 (AF254143) repressed by TUP1 prote... | 106 | 1e-21 |
| ref NP_014458.1 Similar to ferric reductases Fre1p and Fre... | 100 | 1e-19 |
| sp P78588 FREL CANAL PROBABLE FERRIC REDUCTASE TRANSMEMBRAN... | 93 | 2e-17 |
| emb CAB45649.1 (AJ387722) ferric reductase [Candida albicans] | 91 | 6e-17 |
| ref NP_013315.1 Ferric (and cupric) reductase; Fre1p [Sacc... | 91 | 6e-17 |
| ref NP_015029.1 similar to FRE2; Fre5p [Saccharomyces cere... | 87 | 7e-16 |
| ref NP_014489.1 Fre7p [Saccharomyces cerevisiae] >gi 39136... | 68 | 7e-10 |
| ref NP_013049.1 similar to FRE2; Fre6p [Saccharomyces cere... | 67 | 1e-09 |
| sp Q04800 FRP1 SCHPO FERRIC REDUCTASE TRANSMEMBRANE COMPONE... | 55 | 3e-06 |
| pir T40777 ferric reductase transmembrane component - fiss... | 54 | 7e-06 |
| emb CAB91820.1 (AL356192) related to ferric reductase [Neu... | 44 | 0.007 |
| gb AAC39478.1 (AF055356) respiratory burst oxidase protein... | 41 | 0.049 |
| emb CAB70727.1 (AL137404) hypothetical protein [Homo sapiens] | 41 | 0.065 |
| pir S23737 proline-rich protein precursor - kidney bean >g... | 41 | 0.085 |
| gb AAF46035.1 (AE003434) CG15784 gene product [Drosophila ... | 41 | 0.085 |
| ref NP_040968.1 replicase protein (putative); putative [Eg... | 41 | 0.085 |
| pir RRWP6M genome polyprotein - eggplant mosaic virus | 41 | 0.085 |
| pir T01456 extensin homolog F2401.18 - Arabidopsis thalian... | 40 | 0.11 |
| dbj BAA95995.1 (AB040904) KIAA1471 protein [Homo sapiens] | 40 | 0.11 |
| pir T14756 hypothetical protein DKFZp564F0923.1 - human (f... | 40 | 0.15 |
| gb AAB70928.1 (AF020261) proline rich protein [Santalum al... | 39 | 0.25 |
| pir T04859 extensin homolog F28A21.80 - Arabidopsis thalia... | 39 | 0.25 |
| gb AAF87118.1 AC006434.14 (AC006434) F10A5.23 [Arabidopsis ... | 39 | 0.25 |
| pir S51798 gamma-kafirin precursor - sorghum | 39 | 0.25 |

Figure 9A

| | | | |
|---------------------------------------|--|--------------------|------|
| emb CAA44347.1 | (X62480) gamma-kafirin preprotein [Sorghum ... | 39 | 0.25 |
| dbj BAA99921.1 | (AP001306) contains similarity to cell wall... | 38 | 0.43 |
| sp P13983 EXTN TOBAC | EXTENSIN PRECURSOR (CELL WALL HYDROLYP... | 38 | 0.43 |
| ref NP_013148.1 | Ylr047cp [Saccharomyces cerevisiae] >gi 21... | 38 | 0.56 |
| pir T14355 | protein-tyrosine-phosphatase (EC 3.1.3.48) TD14... | 38 | 0.74 |
| gb AAA73078.1 | (M73688) [Sorghum bicolor endosperm tissue m... | 37 | 0.96 |
| sp P48038 ACRO RABIT | ACROSIN PRECURSOR >gi 1085468 pir S47... | 34 | 1.2 |
| pir T00264 | high carbon dioxide response protein 2 - Chloro... | 37 | 1.3 |
| gb AAC82365.1 | (AF055904) unknown [Myxococcus xanthus] | 37 | 1.3 |
| pir A54523 | histidine-rich protein - Plasmodium lophurae (f... | 37 | 1.3 |
| pir T28682 | hypothetical protein - Streptomyces coelicolor ... | 36 | 1.7 |
| pir T06291 | extensin homolog T9E8.80 - Arabidopsis thaliana... | 36 | 1.7 |
| sp P06599 EXTN DAUCA | EXTENSIN PRECURSOR >gi 82047 pir A243... | 36 | 2.2 |
| dbj BAA97321.1 | (AB020754) gene_id:MYN8.5-pir T34137-simil... | 36 | 2.2 |
| pir S25299 | extensin precursor - tomato >gi 170444 gb AAA34... | 36 | 2.2 |
| gb AAF50413.1 | (AE003555) Gug gene product [Drosophila mela... | 36 | 2.8 |
| pir T33997 | hypothetical protein W03G1.5 - Caenorhabditis e... | 36 | 2.8 |
| gb AAD55979.1 | AF159296 1 (AF159296) extensin-like protein [...] | 36 | 2.8 |
| gb AAD41978.1 | AC006438 10 (AC006438) unknown protein (Arabi... | 36 | 2.8 |
| emb CAA76070.1 | (Y16104) overlapping protein [Physalis mott... | 36 | 2.8 |
| gb AAF34752.1 | AF217844 1 (AF217844) GRUNGE [Drosophila mela... | 36 | 2.8 |
| gb AAC39477.1 | (AF055355) respiratory burst oxidase protein... | 35 | 3.7 |
| ref NP_031989.1 | ecotropic viral integration site 1 [Mus mu... | 35 | 3.7 |
| dbj BAB08752.1 | (AB017063) respiratory burst oxidase protei... | 35 | 3.7 |
| gb AAF82153.1 | AC034256 17 (AC034256) Contains similarity to... | 35 | 3.7 |
| dbj BAA21572.1 | (AB002344) KIAA0346 [Homo sapiens] | 35 | 3.7 |
| sp P14404 EVII1 MOUSE | ECOTROPIC VIRUS INTEGRATION 1 SITE PRO... | 35 | 3.7 |
| emb CAB76093.1 | (AL157956) putative oxidoreductase [Strepto... | 35 | 3.7 |
| emb CAA04777.1 | (AJ001482) Evildelta 105 [Mus musculus] | 35 | 3.7 |
| sp P24152 EXTN SORBI | EXTENSIN PRECURSOR (PROLINE-RICH GLYCO... | 35 | 3.7 |
| gb AAF53661.1 | (AE003658) CG15157 gene product [Drosophila ...] | 35 | 4.9 |
| gb AAF21492.1 | U91669 1 (U91669) merozoite surface antigen 2... | 35 | 4.9 |
| gb AAF51119.1 | (AE003580) CG3304 gene product [Drosophila m... | 35 | 4.9 |
| gb AAG21895.1 | AF237962 1 (AF237962) NADH/NADPH thyroid oxid... | 35 | 4.9 |
| sp P20186 YT35 STRFR | HYPOTHETICAL 35.5 KD PROTEIN IN TRANSP... | 35 | 4.9 |
| pir PQ0475 | pistil extensin-like protein (clone pMG04) - co... | 35 | 4.9 |
| gb AAF73291.1 | AF155232 1 (AF155232) extensin [Pisum sativum] | 35 | 4.9 |
| gb AAD37405.1 | AF148222 1 (AF148222) merozoite surface prote... | 35 | 4.9 |
| pir A48232 | cysteine-rich extensin-like protein 1 precursor... | 35 | 4.9 |
| ref NP_057309.1 | Rhd type IIIa protein [Homo sapiens] >gi 6... | 35 | 4.9 |
| gb AAC38842.1 | (AF010462) merozoite surface protein 2 [Plas... | 34 | 6.4 |
| pir T05530 | cytochrome b245 beta chain homolog F13M23.230 -... | 34 | 6.4 |
| gb AAC17609.1 | (AC002131) Strong similarity to extensin-lik... | 34 | 6.4 |
| gb AAA16424.1 | (L13855) UL3.5 [Pseudorabies virus] | 34 | 6.4 |
| gb AAF64002.1 | (AF217011) merozoite surface protein 2 [Plas... | 34 | 6.4 |
| ref XP_001241.1 | similar to hypothetical protein FLJ20337 (...) | 34 | 6.4 |
| gb AAC18092.1 | (AF056965) mutant membrane protein RhCe [Hom... | 34 | 6.4 |
| pir T10863 | extensin precursor - kidney bean >gi 727264 gb ... | 34 | 6.4 |
| emb CAB72300.1 | (AL031284) dJ469D22.1 (Rhesus blood group, ...) | 34 | 6.4 |
| pir B29356 | hydroxyproline-rich glycoprotein precursor (clo... | 34 | 6.4 |
| dbj BAB01951.1 | (AP002048) extensin-like protein [Arabidops... | 34 | 6.4 |
| gb AAF34294.1 | AC005941 6 (AC005941) L5204.7 [Leishmania major] | 34 | 6.4 |
| gb AAD24546.2 | (AF116856) neurocan core protein precursor [...] | 34 | 6.4 |
| pir S40517 | erythrocyte membrane protein - human | 34 | 6.4 |
| ref NP_057208.1 | Rhesus blood group, D antigen; Rhesus syst... | 34 | 6.4 |
| pir S40516 | erythrocyte membrane protein - human | 34 | 6.4 |
| emb CAB09722.1 | (Z97026) rhesus D category VI type III prot... | 34 | 6.4 |
| gb AAD25300.1 | AF088276 1 (AF088276) NADPH oxidase, gp91; ph... | 34 | 8.4 |
| gb AAB34660.1 | RhPI-2e=Rhesus blood group antigen isoform {...} | 34 | 8.4 |
| pir T25800 | C2H2-type zinc finger domain, WT1 homolog - Cae... | 34 | 8.4 |

| | | |
|---|-----------|-----|
| <u>pir</u> <u>S12549</u> hypothetical protein - human herpesvirus 4 | <u>34</u> | 8.4 |
| <u>gb</u> <u>AAF57886.1</u> (AE003804) CG17288 gene product [Drosophila ... | <u>34</u> | 8.4 |
| <u>gb</u> <u>AAD52161.1</u> <u>AF143503.1</u> (AF143503) nitric oxide synthase [... | <u>34</u> | 8.4 |
| <u>dbj</u> <u>BAA35135.1</u> (AB008227) Extensin [Adiantum capillus-vene... | <u>34</u> | 8.4 |
| <u>gb</u> <u>AAB34659.1</u> RhPI-1d=Rhesus blood group antigen isoform {... | <u>34</u> | 8.4 |
| <u>emb</u> <u>CAB65664.1</u> (AJ252251) glycoprotein G-2 [human herpesvi... | <u>34</u> | 8.4 |
| <u>ref</u> <u>NP_044534.1</u> virion glycoprotein G [human herpesvirus 2... | <u>34</u> | 8.4 |
| <u>pir</u> <u>S78480</u> Rhesus blood group antigen-like protein isoform... | <u>34</u> | 8.4 |
| <u>ref</u> <u>NP_005057.1</u> splicing factor proline/glutamine rich (po... | <u>34</u> | 8.4 |
| <u>gb</u> <u>AAF59500.1</u> (AC024805) Hypothetical protein Y51H7C.a [Ca... | <u>34</u> | 8.4 |
| <u>pir</u> <u>I52615</u> gene RhD protein - human >gi 999310 gb AAB34852... | <u>34</u> | 8.4 |
| <u>dbj</u> <u>BAA81899.1</u> (AB018966) Rh blood group D antigen (RhD) [... | <u>34</u> | 8.4 |
| <u>ref</u> <u>NP_065231.1</u> Rhesus blood group, CcEe antigens; Rhesus ... | <u>34</u> | 8.4 |
| <u>pir</u> <u>T29299</u> hypothetical protein C50F7.2 - Caenorhabditis e... | <u>34</u> | 8.4 |

Alignment Report of Untitled, using Clustal method with PAM250 residue weight table.

| | | |
|-----|---|-------------|
| | R Y W V L L C G S I L L C C L S G A S G S T A L T S D Y G K | Majority |
| | 10 20 30 | |
| 1 | R - - - - - L R S A R G T T W L H S D Y I H | Fered.pro |
| 1 | M Y W V L L C G S I L L C C L S G A S A S P A K T K M Y G K | ScFre3p.PRO |
| | L N L V L T D A C T G V L G G A T W E Y S S D D L Y S G S A | Majority |
| | 40 50 60 | |
| 18 | N N I - - - - N C T G P H S G A - - - - - P G S A | Fered.pro |
| 31 | L P L V L T D A C M G V L G E V T W E Y S S D D L Y S S P A | ScFre3p.PRO |
| | C T Y S P A A Q S M L Y C I F E S L A E A G Y S L S G L L K | Majority |
| | 70 80 90 | |
| 34 | P Q Y S P I A Q - - - - - F P P L A P A S M S L S G L L R | Fered.pro |
| 61 | C T Y E P A L Q S M L Y C I Y E S L N E K G Y S N R T F E K | ScFre3p.PRO |
| | S F A A I K E A C A A K T D L L S N W T A A D F Y N M L N N | Majority |
| | 100 110 120 | |
| 58 | S - - - - R E A P A A K R H L L S N W N A A Q F - - - - - | Fered.pro |
| 91 | T F A A I K E D C A Y Y T D N L Q N M T N A D F Y N M L N N | ScFre3p.PRO |
| | G T T Y I I Q Y S E G S A E L T Y S I G L T G V V Q V G N F | Majority |
| | 130 140 150 | |
| 78 | - - - - - E E L K Y S Y G L T G V D Q V G N F | Fered.pro |
| 121 | G T T Y I I Q Y S E G S A N L T Y P I E M D A Q V R E N Y Y | ScFre3p.PRO |
| | L S V D G F L Y A L I G I G H T Y G G I L L A L R V G V M V | Majority |
| | 160 170 180 | |
| 96 | L W V D T F L Y M L I G I S - - - - G M L L M L R I S N M V | Fered.pro |
| 151 | Y S Y H G F - Y A N Y D I G H T Y G G I I C A Y F V G V M I | ScFre3p.PRO |
| | L A S I L H S L A Y G S F K T A L F E T N R L V R Y P W V N | Majority |
| | 190 200 210 | |
| 122 | W K H S R H I T A M G S P R Q K Y W E T N R T S W W P W L N | Fered.pro |
| 180 | L A S I L H Y L S Y T P F K T A L F K - Q R L V R Y - - V R | ScFre3p.PRO |

Figure 9B

Alignment Report of Untitled, using Clustal method with PAM250 residue weight table.

| | | |
|-----|---|-------------|
| | R H L L V A T L W G K K H A A S F S I L S A I T N G T L P G | Majority |
| | 220 230 240 | |
| 152 | R H I L V A P L W K K K H N A Q F Q I S S A I D N G T L P G | Fered.pro |
| 207 | R Y L T I P T I W G K - H A S S F S Y L K I F T - G F L P T | ScFre3p.PRO |
| | R S E G V I L L G Y V G L N V A F C L A L G Y Q Y D P Y D V | Majority |
| | 250 260 270 | |
| 182 | R W H T I M L L I Y V G L N V A W C L A L - - - - P Y D V | Fered.pro |
| 235 | R S E G V I I L G Y L V L H T V F - L A Y G Y Q Y D P Y N L | ScFre3p.PRO |
| | L F D S H R E T L A A Y V A G R S G V L A A A N L I L I A L | Majority |
| | 280 290 300 | |
| 207 | L - - D H R E T L A A - L R G R S G T L A A L N L I P T I L | Fered.pro |
| 264 | I F D S R R E Q I A R Y V A D R S G V L A F A H F P L I A L | ScFre3p.PRO |
| | F A G R N N F L I S L L G V S Y T S F I L F H K W A G R I T | Majority |
| | 310 320 330 | |
| 234 | F A L R N N P L I S L L Q V S Y D D F N L F H R W A A R I T | Fered.pro |
| 294 | F A G R N N F L E F I S G V K Y T S F I M F H K W L G R M M | ScFre3p.PRO |
| | I A D A V V H G A A Y L S N S V A G G G W A A V V A A L H T | Majority |
| | 340 350 360 | |
| 264 | I A E A I V H T A A W L Y N T K A G G G W H A V V A A L H T | Fered.pro |
| 324 | F L D A V I H G A A Y T S Y S V F Y K D W A A S - - - - K | ScFre3p.PRO |
| | E G S Y G W G F G G V A A L T I V G V Q A F F S L A M F R K | Majority |
| | 370 380 390 | |
| 294 | E G S Y G W G M G G T V A F T F I G I Q A W - - - S P F R H | Fered.pro |
| 349 | E E T Y - W Q F G - V A A L C I V G V M V F F S L A M F R K | ScFre3p.PRO |
| | A F Y E A F L N L H R V I V L G A L L G L Y T H L E H V V A | Majority |
| | 400 410 420 | |
| 321 | A F Y E T F L N I H R V M V I A A L L G L Y K H L E - L H A | Fered.pro |
| 377 | F F Y E A F L F L H - - I V L G A L F - F Y T C W E H V V E | ScFre3p.PRO |

Alignment Report of Untitled, usir - Clustal method with PAM250 resid weight table.

| | | |
|-----|---|-------------|
| | L S G V E W I Y A A I A I W A A D R L L R L V S V S Y F G F | Majority |
| | 430 440 450 | |
| 350 | L P Q V P W M Y L I F I F W A A E W F L R L C S I C Y Y G F | Fered.pro |
| 404 | L S G I E W I Y A A I A I W T I D R L I R I V R V S Y F G F | ScFre3p.PRO |
| | S L K Q R S S A S V E A V G G D A V R V T V N R P V R L W T | Majority |
| | 460 470 480 | |
| 380 | S L K Q R S S I T V E A L P G E A V R L T I N M - V R E W T | Fered.pro |
| 434 | P - - - - K A S L Q L V G D D I I R V T V K R P V R L W K | ScFre3p.PRO |
| | A K P G Q H V H V S F L H H L S L W S S H P F S V L D S I I | Majority |
| | 490 500 510 | |
| 409 | P R P G C H V H M - W M P R L S L W S S H P F S V - - - - | Fered.pro |
| 459 | A K P G Q Y V F V S F L H H L Y F W Q S H P F T V L D S I I | ScFre3p.PRO |
| | K D G E L T I I L K E K K G V T K L V K K Y V C C N G G K A | Majority |
| | 520 530 540 | |
| 433 | - | Fered.pro |
| 489 | K D G E L T I I L K E K K G V T K L V K K Y V C C N G G K A | ScFre3p.PRO |
| | S M R L A I E G P Y G S S S P V N N Y D N V L L L T G G T G | Majority |
| | 550 560 570 | |
| 433 | - | Fered.pro |
| 519 | S M R L A I E G P Y G S S S P V N N Y D N V L L L T G G T G | ScFre3p.PRO |
| | L P G P I A H A I K L G K T S A A T G K Q F I K L V I A V R | Majority |
| | 580 590 600 | |
| 433 | - | Fered.pro |
| 549 | L P G P I A H A I K L G K T S A A T G K Q F I K L V I A V R | ScFre3p.PRO |
| | G F N V L E A Y K P E L M C L E D L N V Q L H I Y N T M E A | Majority |
| | 610 620 630 | |
| 433 | - | Fered.pro |
| 579 | G F N V L E A Y K P E L M C L E D L N V Q L H I Y N T M E V | ScFre3p.PRO |

Alignment Report of Untitled, using Clustal method with PAM250 residue weight table.

| | | |
|-----|---|-------------|
| | W A A T P D D S L E I S Q Q D E K A D G K G V V M A T T L E | Majority |
| | 640 650 660 | |
| 434 | W A A T P D R R L - - - - - | Fered.pro |
| 609 | P A L T P N D S L E I S Q Q D E K A D G K G V V M A T T L E | ScFre3p.PRO |
| | Q S D D A V E F G G T V H H D G R P T V E K L L Q E V G T L | Majority |
| | 670 680 690 | |
| 443 | Q R D D A S H F G R R R H H D Q W P T - - - - - Q E I K T N | Fered.pro |
| 639 | Q S P N P V E F D G T V F H H G R P N V E K L L H E V G D L | ScFre3p.PRO |
| | N G S L A V V C C G P P V F V T E V R A Q G A I L V L E K P | Majority |
| | 700 710 720 | |
| 468 | Q S H M P - - C - - - - P Y R T H L R A Q G D I | Fered.pro |
| 669 | N G S L A V V C C G P P V F V D E V R D Q T A N L V L E K P | ScFre3p.PRO |
| | A K A I E Y F E E Y Q S W | Majority |
| | 730 | |
| 485 | | Fered.pro |
| 699 | A K A I E Y F E E Y Q S W | ScFre3p.PRO |

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Figure 9C

CCTTGGTAGTGCACTGGGCGTGTGAATAGCTGTCAGCACGCCCCGCTGGCGGGTTCGCGCCATGGTGGAGATTTTGACAGC
 GACATGGACGACGACGGCCTCGGCAGCGTGAGGAACATGTCAAAATGAACCCAGGGGTGCATCAAAGCCGTTTACCTG
~~AACAGATGAGTGGGATCTCTGCGGGATGGGTGATGAAGTTGACTCGCTTGGACGACGGTTTGGGGGCGAGGCTAGAGCC~~
 GCACATGTTCATCGGCCGGGCATGGCGTCGGGGCCTGCACAGTTCTTGCAGAGAGGGCGCGAAAGAGGGACGAGACAGGC
 GGGTGTGGCGGGCGATAGTGCGGGCGAGAGGGAAAGAGAGTTGGAGAGAGGGAAACCATGGTGTGTTGCTCGCGGCGTCGC
 GCATCGTGCAGGGCGCGTCGGCCCATCATGGCATCGTCATTGGCATCATCGGTACGTACATGGCCGCAAGCATCATGAG
 CACAGCGGCCATTGTCATTTCTCGTGGCGTGGTGGCCGTGGCAAGCTGGTGTCTGTGGCGCGGCGTCTGCGTGTGTGG
 ATCGCCAGGCACCGCGGCGTGATTGGCACAGGCAGGCGAGGGCGGGCATGGATGGATGCAGCGTGTGCAGTCAATCCG
 AAGCACAGCGCAGGGCCGGCAGCCGGCACGTCTGTGGTGGGCGTGTGCAGGCTGGGCGGCGGTGCCGAGTGGGATGGGG
 ATGATAGGATGGATGGGCGAGTCTGGCGAGGGTGGGCTGAGGGGCCCTTGGTTTCATGGCGCTTCCAGGCACGCGGGCCTG
 AGTGGCCAGCATTCGAGCCTCCGCCGCCCGTTTACCCGCTAGCAGCTGGTGGACAGCCCAAGCCAATCGTCCGC
 TGGGCCCTGGCGCCCCCCTGGTGGTGGCGGCCAGGCCACCCCTCGCGCGAACCTCATATCCACTGTTTCCCTCGCT
 CCTCTCCGACCTCACGCTCGCCGTCCCTGCTGTCTGGCTCTCTGCCCACATTGTTTCCACTGTTTCCCTCACTCGCTAC
 GGGAAACCAAAAAACAGCTCCCTGCCCGCTGCTGCTGCTTAACTTACCCACCCCCCGCCGCCGTACACCATTC
 CAAGCCCATTCACCCAGCCGCGCCCTGGCGAGCATCAATCATTTCAACACACAACACTCCCTCCCTCGTTTCATTACG
 CCCGATACTGTGCAGGCCACCCCGCCCTCTGACTCCATTGTGCACACAGCACTGCTGCCCGCCACTTACCATTTGTA
 ATGCATTTCTGTGACCAGCCTGTCTCTCACTCCGCCAACGCCCATCGCTTCGCCTNTCGACTCCGAAGCGCCCGTG
 GGACCACCTGGCTACACAGCGACTACATTCACAACAACATCAACTGCACCGGCCCTCACTCAGGAGCACCTGGTAGCGC
 GCCTCAATACTCGCCCATCGCCCAATTCCCCCTTTAGCACCCGCCAGCATGTCTCTCTCCGGCCTGCTGCGCTCGCGG
 GAGGCACCCGCTGCCAAGCGTCACCTCCTCTCCAAGTGGAAATGCCGCCAGTTTTGAGGAGCTCAAGTACTCGTACGGCC
 TCACTGGTGTGACCAAGTCGGCAACTTCTTGTGGGTGACACCTTTCTCTACATGCTCATTGGCATCTCTGGCATGCT
 CCTCATGCTCCGCATCTCCAACATGGTCTGGAAGCACAGCCGCGCATCACCGCAATGGGAAGCCCAAGGCAAAAGTAC
 TGGGAGACCAACCGAACAAGCTGGTGGCCCTGGCTCAACCGCCACATCTCTCGTCCGCCCGCTCTGGAAGAAGAAGCACA
 ACGCCAGTTCAGATCAGCAGCGGATTGACAACGGAACCTCCCTGGAAGATGGCACACCATCATGCTCTCATCTA
 CGTGGCCCTCAACGTTGCATGGTGCCTTGGCCCTCCCTACGACGTCTCTCGACCACAGGGAGACGCTCGCCGCCCTTCGT
 GGACGCTCTGGAACCTTCGCCGCCCTCAACCTCATCCCCACCATCTCTTCGCCCTCCGCAACAACCCCTCACTCCCT
 TCTCCAGGTCTCGTACGACGACTTCAACCTTTTCCACCGCTGGGCTGCCCGAATCACCATTGCCGAGGCCATTGTCCAC
 ACTGCCGCTTGGTGTGACAACACCAAGGCTGGCGGTGGATGGCACGCCGTCGTAGCTGCCCTCCACACCGAGGGCTCTT
 ACGGATGGGGCATGGGCGGAACCTGTCGCTTACCTTCATCGGCATCCAGGCTGGTCCCATTCGGTCACGCCCTTTTA
 CGAGACCTTTCTCAACATCCACCGCGTCATGGTCATTGCTGCTCTCTCGGCTTGTACAAGCACCTGGAGCTGCACGCT
 CTGCCCCAGGTCCCATGGATGTACCTCATCTTCATCTTCTGGCGGCTGAGTGGTTCTCCGCTGTGCTCCATCTGCT
 ACTACGGCTTCAGCCTGAAGCAACGCTCTTCATACCGTCGAGGCTTGCCTGGCGAAGCTGTCCGTCTAACCATCAA
 CATGGTCCGCGAATGGACCCCCCGTCCCGGATGTACGTGCACATGTGGATGCCCTCGCCTCTCCCTATGGTCTTCGCAT
 CCATTTTCCGTGCGCTGGGCTGCGACCCCTGACCGACGACTCAAAGAGATGACGCTTCCCACTTTGGAAGGCGACGTC
 ACCATGATCAATGGCCAACCCAGGAAATCAAAACAAATCAGTCTCATATGCCGTGCCCGTACCGGACTCACCTTAGAGC
 ACAGGGCGATATTC

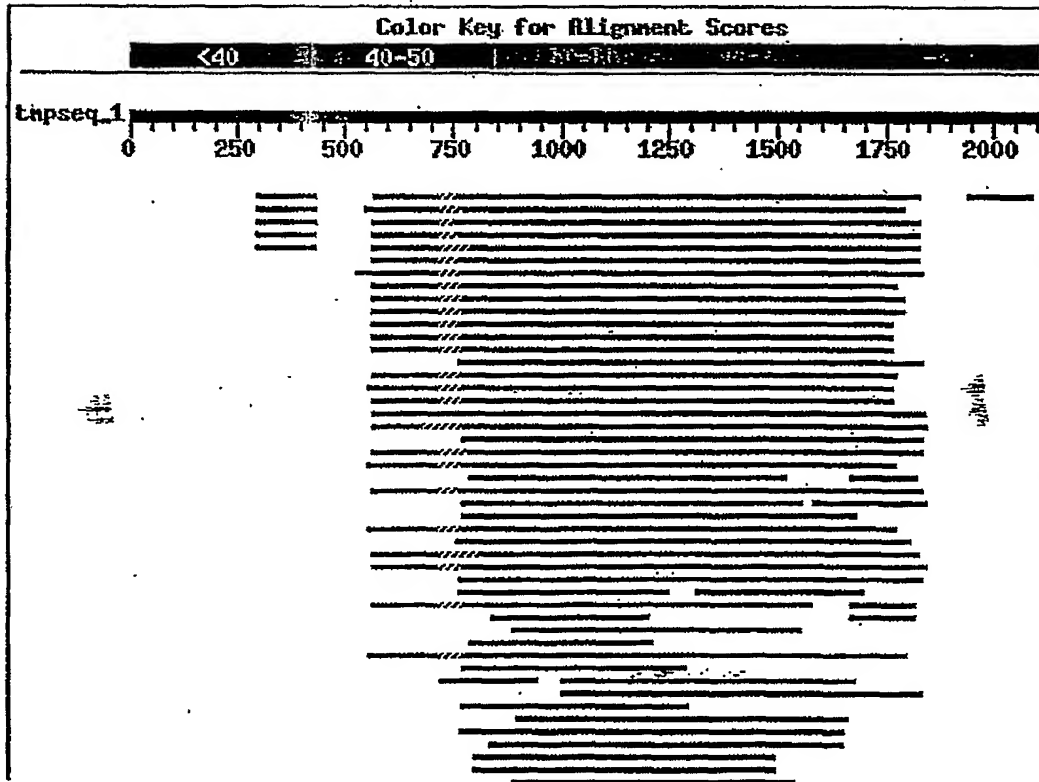
RLRSARGTTWLHSDYIHNNINCTGPHSGAPGSAPQYSPIAQFPPLAPASMSLSGLLSREAPAAKRHLLSNWNAAQFEE
 LKYSYGLTGVDQVGNFLWVDTFLYMLIGISGMLLMRLISNMVWKHSRHI TAMGSPRKQYWE TNRTSWWPWLN RHILVAP
 LWKKKHNAQFQISSAIDNGTLPGRWHTIMLLIYVGLNVAWCLALPYDVLDRHRETLAALRGRSGTLAALNLIPTILFALR
 NNPLISLLQVSYDDFNLFHRWAARITIAEAIVHTAAWLYNTKAGGGWHAVVAALHTEGSYWGMMGTVAFTFIGIQAWS
 PFRHAFYETFLNIHRVMVIAALLGLYKHLELHALPQVPWMYLIFIFWAAEWFLRLCSICYYGFSLKQRSSITVEALPGE
 AVRLTINMVRWETPRPGCHVHMWMPRLSLWSSHPFSVAWAATPDRRLQRDDASHFGRRRHHDQWPTQEIKTNQSHMPCP
 YRTHLRAQGD I

Figure 9D

Figure 10

Distribution of 127 Blast Hits on the Query Sequence

Mouse-over to show defline and scores. Click to show alignments



.../blast.cgi?RID=972605641-1457-10805&DESCRIPTIONS=100&ALIGNMENTS=50&ALIGNMEN10/26/00

Sequences producing significant alignments:

| | Score (bits) | E Value |
|---|-----------------|------------|
| emb CAB99379.1 (AL390354) conserved hypothetical protein [...] | 218 | 7e-65 |
| emb CAB65616.1 (AL136078) probable membrane transporter [S...] | 192 | 1e-57 |
| emb CAB91174.1 (AL355920) putative MFS allantoate permease... | 179 | 4e-55 |
| sp O10097 YAOI SCHPO PUTATIVE TRANSPORTER C11D3.18C >gi 749... | 188 | 1e-53 |
| ref NP_011776.1 Tnalp is a high affinity nicotinic acid pl... | 165 | 4e-49 |
| emb CAB63540.1 (AL133521) putative transporter [Schizosacc...] | 151 | 2e-43 |
| qb AAG07513.1 AE004829_3 (AE004829) probable MFS transporte... | 120 | 3e-31 |
| qb AAG05602.1 AE004648_1 (AE004648) probable MFS transporte... | 116 | 2e-30 |
| pir D64995 hypothetical protein b2246 - Escherichia coli (...) | 116 | 2e-29 |
| sp P76470 YFAV ECOLI HYPOTHETICAL 46.3 KD PROTEIN IN GLPC-A... | 116 | 2e-29 |
| sp P70786 TUB3 AGRVI PUTATIVE TARTRATE TRANSPORTER >gi 9843... | 116 | 6e-29 |
| sp O44470 TUB4 AGRVI PUTATIVE TARTRATE TRANSPORTER >gi 8052... | 114 | 2e-28 |
| emb CAB61275.1 (AL132991) putative transporter protein [St...] | 107 | 6e-27 |
| sp O13880 YB1G SCHPO PUTATIVE TRANSPORTER C1B3.16C >gi 7493... | 121 | 7e-27 |
| emb CAB66219.1 (AL136503) probable transmembrane transport... | 101 | 3e-24 |
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| emb CAA86046.1 (Z37980) hypothetical 4-hydroxyphenylacetat... | 88 | 2e-21 |
| pir T41604 probable membrane transport protein - fission y... | 83 | 3e-21 |
| pir T39680 probable allantoate permease - fission yeast (S... | 91 | 4e-20 |
| qb AAD53495.1 AF144422_2 (AF144422) HpaX [Salmonella dublin] | 80 | 8e-20 |
| ref NP_013104.1 Ylr004cp >gi 2132659 pir S64826 probable ... | 90 | 2e-19 |
| pir T41345 probable allantoate permease - fission yeast (S... | 91 | 4e-17 |
| sp O13879 YE1F SCHPO PUTATIVE TRANSPORTER C1B3.15C >gi 7493... | 91 | 4e-17 |
| ref NP_012686.1 allantoate permease; Dal5p >gi 118233 sp P... | 74 | 1e-16 |
| sp Q05181 PHT1 PSEPU PHTHALATE TRANSPORTER >gi 295708 dbj B... | 87 | 9e-16 |
| pir C70818 probable ABC transporter - Mycobacterium tuberc... | 71 | 3e-15 |
| qb AAD41517.1 AF152094_1 (AF152094) phthalate transporter [...] | 83 | 1e-14 |
| ref NP_009957.1 Amino acid permease; Fen2p >gi 140479 sp P... | 81 | 4e-14 |
| ref NP_009333.1 putative permease; Seolp >gi 731298 sp P39... | 78 | 5e-13 |
| qb AAG05650.1 AE004652_3 (AE004652) probable 2-ketogluconat... | 67 | 5e-13 |
| pir T40485 transmembrane transporter liz1p - fission yeast... | 69 | 2e-10 |
| pir T40140 transmembrane transporter liz1p - fission yeast... | 68 | 4e-10 |
| ref NP_014479.1 Yoll163wp >gi 2132861 pir S66862 probable ... | 63 | 1e-08 |
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| pir T12997 hypothetical protein T21L8.170 - Arabidopsis th... | 50 | 7e-05 |
| dbj BAA13885.1 (D89224) similar to Saccharomyces cerevisia... | 50 | 1e-04 |
| qb AAD46810.1 AF157643_4 (AF157643) putative transporter pr... | 49 | 2e-04 |
| ref NP_005486.1 Na/PO4 cotransporter >gi 4587207 dbj BAA76... | 48 | 4e-04 |
| dbj BAA95074.1 (AB041591) unnamed protein product [Mus mus...] | 47 | 6e-04 |
| sp P39398 YJLJ ECOLI HYPOTHETICAL 49.4 KD PROTEIN IN TSR-MD... | 47 | 6e-04 |
| qb AAD49570.1 AF135037_1 (AF135037) nitrate transporter [Cy...] | 47 | 8e-04 |
| qb AAF55770.1 (AE003730) CG4288 gene product [alt 1] [Dros...] | 46 | 0.002 |
| qb AAD49571.1 (AF135038) nitrate transporter [Cylindrothec...] | 46 | 0.002 |
| qb AAD49572.1 AF135039_1 (AF135039) nitrate transporter [Cy...] | 45 | 0.002 |
| qb AAC13878.1 (U39735) high molecular weight basic nuclear... | 44 | 0.005 |
| sp P31457 DGOT ECOLI D-GALACTONATE TRANSPORTER | 43 | 0.016 |
| pir D65171 hypothetical 48.8 kD protein in ibpA-gyrB inter... | 43 | 0.016 |
| pir T15201 hypothetical protein F12B6.2 - Caenorhabditis e... | 42 | 0.028 |
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| ref NP_011579.1 | H ⁺ -biotin symporter; Vhtlp >gi 1723674 sp ... | 40 | 0.081 |
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| gb AAG03624.1 AE004461_7 | (AE004461) 4-hydroxybenzoate trans... | 38 | 0.31 |
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| gb AAB59301.1 | (L20696) meiotin-1 [Lilium longiflorum] | 38 | 0.54 |
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| ref NP_037488.1 | monocarboxylate transporter 3 >gi 6093322 ... | 37 | 0.71 |
| pir T07796 | DNA-directed RNA polymerase (EC 2.7.7.6) larges... | 37 | 0.71 |
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| sp Q28722 NPT1_RABIT | RENAL SODIUM-DEPENDENT PHOSPHATE TRANS... | 36 | 1.2 |
| pir F75580 | probable sugar transporter - Deinococcus radiod... | 36 | 1.2 |
| pir S56583 | hypothetical protein f261b - Escherichia coli >... | 36 | 1.6 |
| sp Q01636 LMBV_CHICK | LAMININ BETA-1 CHAIN VARIANT (LAMININ ... | 36 | 2.1 |
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| gb AAF44801.1 AE003406_6 | (AE003416) hypothetical protein [D... | 35 | 2.7 |
| gb AAF67524.1 AF204396_1 | (AF204396) monocarboxylate transpo... | 35 | 2.7 |
| sp Q62795 NPT1_RAT | RENAL SODIUM-DEPENDENT PHOSPHATE TRANSPO... | 35 | 3.6 |
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| ref NP_036566.1 | solute carrier family 17 (anion/sugar tran... | 35 | 3.6 |
| gb AAC15775.1 | (AF061335) oxytetracycline exporter [Strepto... | 34 | 4.7 |
| pir JE0378 | DNA (cytosine-5-)-methyltransferase (EC 2.1.1.3... | 34 | 4.7 |
| pir F69443 | octaprenyl-diphosphate synthase (ispB) homolog ... | 34 | 4.7 |
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| emb CAB76602.1 | (AJ271264) glycerol kinase [Staphylococcus ... | 34 | 4.7 |
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| gb AAB28462.1 | extensin-nodule-specific proline-rich protei... | 34 | 6.1 |
| emb CAB67228.1 | (AJ271079) NdhF' protein [Oenothera elata s... | 34 | 6.1 |
| emb CAB67217.1 | (AJ271079) NADH-plastoquinone oxidoreductas... | 34 | 6.1 |
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| emb CAB76609.1 | (AJ271271) glycerol kinase [Staphylococcus ... | 34 | 6.1 |
| ref NP_009852.1 | Probable multidrug resistance protein; Ybr... | 34 | 8.0 |
| pir T27092 | hypothetical protein Y51B9A.6 - Caenorhabditis ... | 34 | 8.0 |
| sp Q51955 PCAK_PSEPU | 4-HYDROXYBENZOATE TRANSPORTER >gi 1147... | 34 | 8.0 |
| pir T34995 | probable integral membrane efflux protein - Str... | 34 | 8.0 |
| gb AAC69842.1 | (AF076683) unknown [Staphylococcus aureus] | 34 | 8.0 |
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SEQUENCE LISTING

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5 Cornell Research Foundation, Inc.
 Yoder, Olen
 Turgeon, Barbara G.
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| tcac | ac | gg | ga | gat | gc | gaca | atag | ccc | agt | ac | agt | tata | cg | gg | at | ccta | at | gg | ac | agg | att | | | 5340 | | | | | |
| ccc | ag | at | gat | cac | gga | ag | ag | cct | ggt | cc | ac | ggg | ctag | cat | gact | gga | agt | at | g | ctt | ggg | c | | 5400 | | | | | |
| 20ga | at | ggg | cg | ccc | ag | ccag | t | at | ca | agg | ccg | gg | t | cg | aca | ag | ag | cacc | gag | ct | aat | ggg | ga | 5460 | | | | | |
| tgac | ag | cg | gac | tat | ga | ata | at | ctat | cc | ctt | a | cac | ag | cag | ca | ac | ag | cag | caa | tac | ca | ac | ag | c | 5520 | | | | |
| cg | gt | at | gta | tg | ct | ca | ac | ag | ca | agg | cat | gc | ac | ccc | ag | ca | aca | ac | ccaa | tt | tag | cat | gt | | 5580 | | | | |
| cca | ac | ac | g | cc | aca | agg | t | cc | ac | ccc | aa | g | gc | g | tag | aa | act | ac | at | gat | cc | at | ag | cg | acc | gca | 5640 | | |
| cacca | ac | aga | ca | ac | cc | gg | cac | t | ctt | t | cct | tg | ccg | ac | cc | cg | cg | tat | gc | aga | ac | cagg | g | ccaaa | | 5700 | | | |
| 25tg | aac | gag | ac | ggg | gc | ct | ac | ga | ac | cc | at | ga | act | at | ca | aaa | cg | cg | tat | cat | cc | g | cat | ca | ac | | 5760 | | |
| aaca | ata | cg | a | at | ct | ga | ag | ac | ggg | ggg | ag | ca | gact | cag | cg | gg | cccc | gt | gcca | gac | gt | g | ct | g | tc | | 5820 | | |
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| tgt | gga | ata | a | tc | gc | gag | tac | tat | gg | ta | aca | a | gccc | at | cg | ta | tgc | agg | cg | ga | tac | ac | g | ca | ag | | 5940 | | |
| at | gg | ca | at | at | cc | ac | gag | cag | ca | aca | ac | ac | g | at | gag | tac | ac | gag | ta | at | g | cg | t | cat | at | gg | cg | 6000 | |
| 30gaa | at | ca | agg | ag | cagg | cg | ga | gg | cag | cg | gg | cg | g | cg | gt | gg | cg | t | ctcc | gag | tt | g | caa | at | cg | t | | 6060 | |
| acag | ct | cc | ga | cag | cg | agg | gt | gc | ag | t | gac | g | ac | g | ctt | gg | ag | ac | gt | gat | g | cc | tt | g | ct | ca | ga | 6120 | |
| tca | at | ttt | tg | ggg | cg | cg | ct | g | ct | g | ct | gc | ct | cg | tg | gag | ac | ct | g | ct | g | ct | g | g | t | g | ctt | ctt | 6180 |
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| acat | ag | ag | ac | cg | tt | gt | ata | c | gc | agg | ttt | ca | aatt | aga | aga | gc | ga | aat | at | g | c | at | at | cag | ct | g | | | 6300 |
| 35tt | gtt | tca | at | g | tt | ctag | ttt | g | ga | agg | gt | taa | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | cccc | 6360 |
| act | t | gtt | tt | gt | gt | gt | att | ta | aat | ct | gg | gaga | ttt | caa | at | ct | ac | at | ct | cg | ct | ata | cat | ag | gt | | | | 6420 |
| gtt | gtt | tg | at | aac | g | tag | ggg | g | caga | agg | gt | at | ct | cg | t | gat | att | tag | act | gg | gag | tt | g | cat | g | | | | 6480 |
| aat | ca | agg | tg | tt | gag | caaaa | aa | ag | ag | ag | ag | cg | gt | ga | agg | gg | cg | gg | gg | gg | gg | gg | gg | gg | gg | gg | gg | gg | 6540 |
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      20              25              30
    Gly Asp Ala Phe Ser Pro Thr Ala Ala Ile Pro Pro Pro Met Met Asn
      35              40              45
    Pro Asn Asp Leu Pro Leu Gly Ala Ala Glu Thr Met Met Pro Leu Glu
15    50              55              60
    Pro Arg Asp Leu Pro Phe Asp Val Tyr Asp Pro His Asn Pro Asn Val
    65              70              75              80
    Lys Met Ser Lys Phe Asp Asn Ile Gly Ala Val Leu Arg His Arg Ser
      85              90              95
20Arg Thr Gln Pro Arg Thr Thr Ala Phe Trp Val Leu Asp Ala Lys Gly
      100              105              110
    Lys Glu Thr Ala Ser Ile Thr Trp Glu Lys Val Ala Ser Arg Ala Glu
      115              120              125
    Lys Val Ala Lys Val Ile Arg Asp Lys Ser Asn Leu Tyr Arg Gly Asp
25    130              135              140
    Arg Val Ala Leu Val Tyr Arg Asp Thr Glu Ile Ile Asp Phe Val Val
    145              150              155              160
    Ala Leu Met Gly Cys Phe Ile Ala Gly Val Val Ala Val Pro Ile Asn
      165              170              175
30Ser Val Asp Asp Tyr Gln Lys Leu Ile Leu Leu Leu Thr Thr Thr Gln
      180              185              190
    Ala His Leu Ala Leu Thr Thr Asp Asn Asn Leu Lys Ala Phe His Arg
      195              200              205
    Asp Ile Ser Gln Asn Arg Leu Lys Trp Pro Ser Gly Val Glu Trp Trp
35    210              215              220
    Lys Thr Asn Glu Phe Gly Ser His His Pro Lys Lys His Asp Asp Thr
    225              230              235              240
    Pro Ala Leu Gln Val Pro Glu Val Ala Tyr Ile Glu Phe Ser Arg Ala
      245              250              255
40Pro Thr Gly Asp Leu Arg Gly Val Val Leu Ser His Arg Thr Ile Met

```

| | | | | | |
|----|---|--|-----|--|-----|
| | 260 | | 265 | | 270 |
| | His Gln Met Ala Cys Ile Ser Ala Met Ile Ser Thr Ile Pro Thr Asn | | | | |
| | 275 | | 280 | | 285 |
| | Ala Gln Ser Gln Asp Thr Phe Ser Thr Ser Leu Arg Asp Ala Glu Gly | | | | |
| 5 | 290 | | 295 | | 300 |
| | Lys Phe Val Ala Pro Ala Pro Ser Arg Asn Pro Thr Glu Val Ile Leu | | | | |
| | 305 | | 310 | | 315 |
| | Thr Tyr Leu Asp Pro Arg Glu Ser Ala Gly Leu Ile Leu Ser Val Leu | | | | |
| | 325 | | 330 | | 335 |
| 10 | Phe Ala Val Tyr Gly Gly His Thr Thr Val Trp Leu Glu Thr Ala Thr | | | | |
| | 340 | | 345 | | 350 |
| | Met Glu Thr Pro Gly Leu Tyr Ala His Leu Ile Thr Lys Tyr Lys Ser | | | | |
| | 355 | | 360 | | 365 |
| | Asn Ile Leu Leu Ala Asp Tyr Pro Gly Leu Lys Arg Ala Ala Tyr Asn | | | | |
| 15 | 370 | | 375 | | 380 |
| | Tyr Gln Gln Asp Pro Met Ala Thr Arg Asn Phe Lys Lys Asn Thr Glu | | | | |
| | 385 | | 390 | | 395 |
| | Pro Asn Phe Ala Ser Val Lys Ile Cys Leu Ile Asp Thr Leu Thr Val | | | | |
| | 405 | | 410 | | 415 |
| 20 | Asp Cys Glu Phe His Glu Ile Leu Gly Asp Arg Tyr Phe Arg Pro Leu | | | | |
| | 420 | | 425 | | 430 |
| | Arg Asn Pro Arg Ala Arg Glu Leu Ile Ala Pro Met Leu Cys Leu Pro | | | | |
| | 435 | | 440 | | 445 |
| | Glu His Gly Gly Met Ile Ile Ser Val Arg Asp Trp Leu Gly Gly Glu | | | | |
| 25 | 450 | | 455 | | 460 |
| | Glu Arg Met Gly Cys Pro Leu Ser Ile Ala Val Glu Glu Ser Asp Asn | | | | |
| | 465 | | 470 | | 475 |
| | Asp Glu Asp Asp Thr Glu Asp Lys Tyr Ala Ala Ala Asn Gly Tyr Ser | | | | |
| | 485 | | 490 | | 495 |
| 30 | Ser Leu Ile Gly Gly Gly Thr Thr Lys Asn Lys Lys Glu Lys Lys Lys | | | | |
| | 500 | | 505 | | 510 |
| | Lys Gly Pro Thr Glu Leu Thr Glu Ile Leu Leu Asp Lys Glu Ala Leu | | | | |
| | 515 | | 520 | | 525 |
| | Lys Met Asn Glu Val Ile Val Leu Ala Ile Gly Glu Glu Ala Ser Lys | | | | |
| 35 | 530 | | 535 | | 540 |
| | Arg Ala Asn Glu Pro Gly Thr Met Arg Val Gly Ala Phe Gly Tyr Pro | | | | |
| | 545 | | 550 | | 555 |
| | Ile Pro Asp Ala Thr Leu Ala Ile Val Asp Pro Glu Thr Ser Leu Leu | | | | |
| | 565 | | 570 | | 575 |
| 40 | Cys Ser Pro Tyr Ser Ile Gly Glu Ile Trp Val Asp Ser Pro Ser Leu | | | | |

| | | | | | |
|----|---|--|-----|--|-----|
| | 580 | | 585 | | 590 |
| | Ser Gly Gly Phe Trp Gln Leu Gln Lys His Thr Glu Thr Ile Phe His | | | | |
| | 595 | | 600 | | 605 |
| | Ala Arg Pro Tyr Arg Phe Val Glu Gly Ser Pro Thr Pro Gln Leu Leu | | | | |
| 5 | 610 | | 615 | | 620 |
| | Glu Leu Glu Phe Leu Arg Thr Gly Leu Leu Gly Phe Val Val Glu Gly | | | | |
| | 625 | | 630 | | 635 |
| | Lys Ile Phe Val Leu Gly Leu Tyr Glu Asp Arg Ile Arg Gln Arg Val | | | | |
| | 645 | | 650 | | 655 |
| 10 | Glu Trp Val Glu Asn Gly Gln Leu Glu Ala Glu His Arg Tyr Phe Phe | | | | |
| | 660 | | 665 | | 670 |
| | Val Gln His Leu Val Thr Ser Ile Met Lys Ala Val Pro Lys Ile Tyr | | | | |
| | 675 | | 680 | | 685 |
| | Asp Cys Ser Ser Phe Asp Ser Tyr Val Asn Gly Glu Tyr Leu Pro Ile | | | | |
| 15 | 690 | | 695 | | 700 |
| | Ile Leu Ile Glu Thr Gln Ala Ala Ser Thr Ala Pro Thr Asn Pro Gly | | | | |
| | 705 | | 710 | | 715 |
| | Gly Pro Pro Gln Gln Leu Asp Ile Pro Phe Leu Asp Ser Leu Ser Glu | | | | |
| | 725 | | 730 | | 735 |
| 20 | Arg Cys Met Glu Val Leu Tyr Gln Glu His His Leu Arg Val Tyr Cys | | | | |
| | 740 | | 745 | | 750 |
| | Val Met Ile Thr Ala Pro Asn Thr Leu Pro Arg Val Ile Lys Asn Gly | | | | |
| | 755 | | 760 | | 765 |
| | Arg Arg Glu Ile Gly Asn Met Leu Cys Arg Arg Glu Phe Asp Asn Gly | | | | |
| 25 | 770 | | 775 | | 780 |
| | Ser Leu Pro Cys Val His Val Lys Phe Gly Ile Glu Arg Ser Val Gln | | | | |
| | 785 | | 790 | | 795 |
| | Asn Ile Ala Leu Gly Asp Asp Pro Ala Gly Gly Met Trp Ser Phe Glu | | | | |
| | 805 | | 810 | | 815 |
| 30 | Ala Ser Met Ala Arg Gln Gln Phe Leu Met Leu Gln Asp Lys Gln Tyr | | | | |
| | 820 | | 825 | | 830 |
| | Ser Gly Val Asp His Arg Glu Val Val Ile Asp Asp Arg Thr Ser Thr | | | | |
| | 835 | | 840 | | 845 |
| | Pro Leu Asn Gln Phe Ser Asn Ile His Asp Leu Met Gln Trp Arg Val | | | | |
| 35 | 850 | | 855 | | 860 |
| | Ser Arg Gln Ala Glu Glu Leu Ala Tyr Cys Thr Val Asp Gly Arg Gly | | | | |
| | 865 | | 870 | | 875 |
| | Lys Glu Gly Lys Gly Val Asn Trp Lys Lys Phe Asp Gln Lys Val Ala | | | | |
| | 885 | | 890 | | 895 |
| 40 | Gly Val Ala Met Tyr Leu Lys Asn Lys Val Lys Val Gln Ala Gly Asp | | | | |

| | | | | | |
|----|---|-------------------------------------|------|------|-----|
| | 900 | | 905 | | 910 |
| | His Leu Leu Leu Met Tyr Thr | His Ser Glu Glu Phe Val Tyr Ala Val | | | |
| | 915 | 920 | 925 | | |
| | His Ala Cys Phe Val Leu Gly Ala Val Cys Ile Pro Met Ala Pro Ile | | | | |
| 5 | 930 | 935 | 940 | | |
| | Asp Gln Asn Arg Leu Asn Glu Asp Ala Pro Ala Leu Leu His Ile Leu | | | | |
| | 945 | 950 | 955 | 960 | |
| | Ala Asp Phe Lys Val Lys Ala Ile Leu Val Asn Ala Asp Val Asp His | | | | |
| | 965 | 970 | 975 | | |
| 10 | Leu Met Lys Ile Lys Gln Val Ser Gln His Ile Lys Gln Ser Ala Ala | | | | |
| | 980 | 985 | 990 | | |
| | Ile Leu Lys Ile Ser Val Pro Asn Thr Tyr Ser Thr Thr Lys Pro Pro | | | | |
| | 995 | 1000 | 1005 | | |
| | Lys Gln Ser Ser Gly Cys Arg Asp Leu Lys Leu Thr Ile Arg Pro Ala | | | | |
| 15 | 1010 | 1015 | 1020 | | |
| | Trp Ile Gln Ala Gly Phe Pro Val Leu Val Trp Thr Tyr Trp Thr Pro | | | | |
| | 1025 | 1030 | 1035 | 1040 | |
| | Asp Gln Arg Arg Ile Ala Val Gln Leu Gly His Ser Gln Ile Met Ala | | | | |
| | 1045 | 1050 | 1055 | | |
| 20 | Leu Cys Lys Val Gln Lys Glu Thr Cys Gln Met Thr Ser Thr Arg Pro | | | | |
| | 1060 | 1065 | 1070 | | |
| | Val Leu Gly Cys Val Arg Ser Thr Ile Gly Leu Gly Phe Leu His Thr | | | | |
| | 1075 | 1080 | 1085 | | |
| | Cys Leu Met Gly Ile Phe Leu Ala Ala Pro Thr Tyr Leu Val Ser Pro | | | | |
| 25 | 1090 | 1095 | 1100 | | |
| | Val Asp Phe Ala Gln Asn Pro Asn Ile Leu Phe Gln Thr Leu Ser Arg | | | | |
| | 1105 | 1110 | 1115 | 1120 | |
| | Tyr Lys Ile Lys Asp Ala Tyr Ala Thr Ser Gln Met Leu Asp His Ala | | | | |
| | 1125 | 1130 | 1135 | | |
| 30 | Ile Ala Arg Gly Ala Gly Lys Ser Met Ala Leu His Glu Leu Lys Asn | | | | |
| | 1140 | 1145 | 1150 | | |
| | Leu Met Ile Ala Thr Asp Gly Arg Pro Arg Val Asp Val Tyr Gln Arg | | | | |
| | 1155 | 1160 | 1165 | | |
| | Val Arg Val His Phe Ala Pro Ala Asn Leu Asp Pro Thr Ala Ile Asn | | | | |
| 35 | 1170 | 1175 | 1180 | | |
| | Thr Val Tyr Ser His Val Leu Asn Pro Met Val Ala Ser Arg Ser Tyr | | | | |
| | 1185 | 1190 | 1195 | 1200 | |
| | Met Cys Ile Glu Pro Val Glu Leu His Leu Asp Val His Ala Leu Arg | | | | |
| | 1205 | 1210 | 1215 | | |
| 40 | Arg Gly Leu Val Met Pro Val Asp Pro Asp Thr Glu Pro Asn Ala Leu | | | | |

| | | | |
|----|---|------|------|
| | 1220 | 1225 | 1230 |
| | Leu Val Gln Asp Ser Gly Met Val Pro Val Ser Thr Gln Ile Ser Ile | | |
| | 1235 | 1240 | 1245 |
| | Val Asn Pro Glu Thr Asn Gln Leu Cys Leu Asn Gly Glu Tyr Gly Glu | | |
| 5 | 1250 | 1255 | 1260 |
| | Ile Trp Val Gln Ser Glu Ala Asn Ala Tyr Ser Phe Tyr Met Ser Lys | | |
| | 1265 | 1270 | 1275 |
| | Glu Arg Leu Asp Ala Glu Arg Phe Asn Gly Arg Thr Ile Asp Gly Asp | | 1280 |
| | 1285 | 1290 | 1295 |
| 10 | Pro Asn Val Arg Tyr Val Arg Thr Gly Asp Leu Gly Phe Leu His Ser | | |
| | 1300 | 1305 | 1310 |
| | Val Thr Arg Pro Ile Gly Pro Asn Gly Ala Pro Val Asp Met Gln Val | | |
| | 1315 | 1320 | 1325 |
| | Leu Phe Val Leu Gly Ser Ile Gly Asp Thr Phe Glu Val Asn Gly Leu | | |
| 15 | 1330 | 1335 | 1340 |
| | Asn His Phe Ser Met Asp Ile Glu Gln Ser Val Glu Arg Cys His Arg | | |
| | 1345 | 1350 | 1355 |
| | Asn Ile Val Pro Gly Gly Cys Ala Val Phe Gln Ala Gly Gly Leu Val | | |
| | 1365 | 1370 | 1375 |
| 20 | Val Val Val Val Glu Ile Phe Arg Arg Asn Phe Leu Ala Ser Met Val | | |
| | 1380 | 1385 | 1390 |
| | Pro Val Ile Val Asn Ala Ile Leu Asn Glu His Gln Leu Val Ile Asp | | |
| | 1395 | 1400 | 1405 |
| | Ile Val Ser Phe Val Gln Lys Gly Asp Phe His Arg Ser Arg Leu Gly | | |
| 25 | 1410 | 1415 | 1420 |
| | Glu Lys Gln Arg Gly Lys Ile Leu Ala Gly Trp Val Thr Arg Lys Met | | |
| | 1425 | 1430 | 1435 |
| | Arg Thr Ile Ala Gln Tyr Ser Ile Arg Asp Pro Asn Gly Gln Asp Ser | | |
| | 1445 | 1450 | 1455 |
| 30 | Gln Met Ile Thr Glu Glu Pro Gly Pro Arg Ala Ser Met Thr Gly Ser | | |
| | 1460 | 1465 | 1470 |
| | Met Leu Gly Arg Met Gly Gly Pro Ala Ser Ile Lys Ala Gly Ser Thr | | |
| | 1475 | 1480 | 1485 |
| | Arg Ala Pro Ser Leu Met Gly Met Thr Ala Thr Met Asn Asn Leu Ser | | |
| 35 | 1490 | 1495 | 1500 |
| | Leu Thr Gln Gln Gln Gln Gln Gln Tyr Gln Gln Pro Gly Met Tyr Ala | | |
| | 1505 | 1510 | 1515 |
| | Gln Gln Gln Gly Met His Pro Gln Gln Gln His Gln Phe Ser Met Ser | | 1520 |
| | 1525 | 1530 | 1535 |
| 40 | Asn Thr Pro Pro Gln Gly Pro Pro Gln Gly Val Glu Leu His Asp Pro | | |

10

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Ser Asp Arg Thr Pro Thr Asp Asn Arg His Ser Phe Leu Ala Asp Pro
1555          1560          1565
Arg Met Gln Asn Gln Gly Gln Met Asn Glu Thr Gly Ala Tyr Glu Pro
5  1570          1575          1580
Met Asn Tyr Gln Asn Ala Tyr His Pro His Gln Gln Gln Tyr Glu Ser
1585          1590          1595          1600
Glu Asp Gly Gly Ser Arg Leu Ser Gly Pro Val Pro Asp Val Leu Arg
1605          1610          1615
10Pro Gly Pro Ser Ser Gly Ser Ile Glu Gln His Asp Gln Ala Asn Asn
1620          1625          1630
Asp Asn Asn Met Trp Asn Asn Arg Glu Tyr Tyr Gly Asn Ser Pro Ser
1635          1640          1645
Tyr Ala Gly Gly Tyr Thr Gln Asp Gly Asn Ile His Glu Gln Gln Gln
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His Asp Glu Tyr Thr Ser Asn Ala Ser Tyr Gly Gly Asn Gln Gly Ala
1665          1670          1675          1680
Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Leu Arg Val Ala Asn Arg Asp
1685          1690          1695
20Ser Ser Asp Ser Glu Gly Ala Asp Asp Asp Ala Trp Arg Arg Asp Ala
1700          1705          1710
Leu Ala Gln Ile Asn Phe Ala Gly Gly Ala Ala Ala Ala Ser Ala Gly
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23

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 Gln Gln Gln Gln Gln Tyr Asp Gly Gln Gln Tyr Asp Gly Arg Thr Thr
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 His Tyr Asp Gln Thr Gln Thr Val Glu Tyr Val Gly Pro Gln Gln Arg
 65 70 75 80
 Tyr Ser Ser Ser Asp Ala Phe Ser Pro Thr Ala Ala Met Ala Pro Pro
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 Tyr Arg Gly Asp Arg Val Ala Leu Ile Tyr Arg Asp Ser Glu Val Ile
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24

| | | | | | |
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| | 225 | | 230 | | 235 |
| | Thr Thr Thr Gln Ala His Leu Ala Leu Thr Thr Asp Asn Asn Leu Lys | | | | |
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| | Ala Phe Gln Arg Asp Ile Thr Thr Gln Lys Leu Thr Trp Pro Lys Gly | | | | |
| | | 260 | | 265 | 270 |
| | Val Glu Trp Trp Lys Thr Asn Glu Phe Gly Ser Tyr His Pro Lys Lys | | | | |
| | 275 | | 280 | | 285 |
| 10 | Lys Glu Asp Val Pro Ala Leu Val Val Pro Asp Leu Ala Tyr Ile Glu | | | | |
| | 290 | | 295 | | 300 |
| | Phe Ser Arg Ala Pro Thr Gly Asp Leu Arg Gly Val Val Leu Ser His | | | | |
| | 305 | | 310 | | 315 |
| | Arg Thr Ile Met His Gln Met Ala Cys Leu Ser Ala Ile Ile Ser Thr | | | | |
| 15 | | 325 | | 330 | 335 |
| | Ile Pro Gly Asn Gly Pro Gly Asp Thr Phe Asn Pro Ser Leu Arg Asp | | | | |
| | | 340 | | 345 | 350 |
| | Lys Asn Gly Arg Leu Ile Gly Gly Gly Ala Ser Ser Glu Ile Leu Val | | | | |
| | 355 | | 360 | | 365 |
| 20 | Ser Tyr Leu Asp Pro Arg Gln Gly Ile Gly Met Ile Leu Ser Val Leu | | | | |
| | 370 | | 375 | | 380 |
| | Leu Thr Val Tyr Gly Gly His Thr Thr Val Trp Phe Asp Asn Lys Ala | | | | |
| | 385 | | 390 | | 395 |
| | Val Asp Val Pro Gly Leu Tyr Ala His Leu Leu Thr Lys Tyr Lys Ser | | | | |
| 25 | | 405 | | 410 | 415 |
| | Thr Ile Met Ile Ala Asp Tyr Pro Gly Leu Lys Arg Ala Ala Tyr Asn | | | | |
| | | 420 | | 425 | 430 |
| | Tyr Gln Gln Glu Pro Met Val Thr Arg Asn Phe Lys Lys Gly Met Glu | | | | |
| | 435 | | 440 | | 445 |
| 30 | Pro Asn Phe Gln Met Ile Lys Leu Cys Leu Ile Asp Thr Leu Thr Val | | | | |
| | 450 | | 455 | | 460 |
| | Asp Ser Gly Ser His Glu Val Leu Ala Asp Arg Trp Leu Arg Pro Leu | | | | |
| | 465 | | 470 | | 475 |
| | Arg Asn Pro Arg Ala Arg Glu Val Val Ala Pro Met Leu Cys Leu Pro | | | | |
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| | Glu His Gly Gly Met Val Ile Ser Val Arg Asp Trp Leu Gly Gly Glu | | | | |
| | | 500 | | 505 | 510 |
| | Glu Arg Met Gly Cys Pro Leu Lys Leu Glu Leu Gly Glu Asp Thr Glu | | | | |
| | 515 | | 520 | | 525 |
| 40 | Ser Asp Glu Glu Lys Glu Glu Thr Glu Lys Pro Ala Val Ser Asn Gly | | | | |

25

| | | |
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| Phe Gly Ser Leu Leu Ser Gly Gly Gly Thr Ala Thr Thr Glu Glu Arg | | |
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| Ala Lys Asn Glu Leu Gly Glu Val Leu Leu Asp Arg Glu Ala Leu Lys | | |
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| Thr Asn Glu Val Val Val Val Ala Ile Gly Asn Asp Ala Arg Lys Arg | | |
| | 580 | 585 |
| Val Thr Asp Asp Pro Gly Leu Val Arg Val Gly Ser Phe Gly Tyr Pro | | |
| | 595 | 600 |
| 10 Ile Pro Asp Ala Thr Leu Ser Val Val Asp Pro Glu Thr Gly Leu Leu | | |
| | 610 | 615 |
| Ala Ser Pro His Ser Val Gly Glu Ile Trp Val Asp Ser Pro Ser Leu | | |
| | 625 | 630 |
| Ser Gly Gly Phe Trp Ala Gln Pro Lys Asn Thr Glu Leu Ile Phe His | | |
| 15 | 645 | 650 |
| Ala Arg Pro Tyr Lys Phe Asp Pro Gly Asp Pro Thr Pro Gln Pro Val | | |
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| Glu Pro Glu Phe Leu Arg Thr Gly Leu Leu Gly Thr Val Ile Glu Gly | | |
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| | 690 | 695 |
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| Asp Cys Ser Ala Phe Asp Val Phe Val Asn Asp Glu His Leu Pro Val | | |
| | 740 | 745 |
| Val Val Leu Glu Ser Ala Ala Ala Ser Thr Ala Pro Leu Thr Ser Gly | | |
| | 755 | 760 |
| 30 Gly Pro Pro Arg Gln Pro Asp Thr Ala Leu Leu Glu Ser Leu Ala Glu | | |
| | 770 | 775 |
| Arg Cys Met Glu Val Leu Met Ser Glu His His Leu Arg Leu Tyr Cys | | |
| | 785 | 790 |
| Val Met Ile Thr Ala Pro Asp Thr Leu Pro Arg Val Val Lys Asn Gly | | |
| 35 | 805 | 810 |
| Arg Arg Glu Ile Gly Asn Met Leu Cys Arg Arg Glu Phe Asp Leu Gly | | |
| | 820 | 825 |
| Asn Leu Pro Cys Val His Val Lys Phe Gly Val Glu His Ala Val Leu | | |
| | 835 | 840 |
| 40 Asn Leu Pro Ile Gly Val Asp Pro Ile Gly Gly Ile Trp Ser Pro Leu | | |

| | | | | | | | | | | | | | | | | | | | |
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| | 850 | | 855 | | 860 | | | | | | | | | | | | | | |
| | Ala | Ser | Asp | Ser | Arg | Ala | Glu | Phe | Leu | Leu | Pro | Ala | Asp | Lys | Gln | Tyr | | | |
| | 865 | | | | | 870 | | | | | 875 | | | | | 880 | | | |
| | Ser | Gly | Val | Asp | Arg | Arg | Glu | Val | Val | Ile | Asp | Asp | Arg | Thr | Ser | Thr | | | |
| 5 | | | | | 885 | | | | | 890 | | | | | 895 | | | | |
| | Pro | Leu | Asn | Asn | Phe | Ser | Cys | Ile | Ser | Asp | Leu | Ile | Gln | Trp | Arg | Val | | | |
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| | Ala | Arg | Gln | Pro | Glu | Glu | Leu | Ala | Tyr | Cys | Thr | Ile | Asp | Gly | Lys | Ser | | | |
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| | 945 | | | | 950 | | | | 955 | | | | 960 | | | | | | |
| | His | Ile | Ile | Leu | Met | Tyr | Thr | His | Ser | Glu | Glu | Phe | Val | Phe | Ala | Ile | | | |
| 15 | | | | 965 | | | | 970 | | | | 975 | | | | | | | |
| | His | Ala | Cys | Ile | Ser | Leu | Gly | Ala | Ile | Val | Ile | Pro | Ile | Ala | Pro | Leu | | | |
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| | Val | Leu | Lys | Ile | Thr | Ser | Pro | Ala | Ile | Tyr | Asn | Thr | Thr | Lys | Pro | Pro | | | |
| 25 | | | 1045 | | | | 1050 | | | | 1055 | | | | | | | | |
| | Lys | Gln | Ser | Ser | Gly | Leu | Arg | Asp | Leu | Arg | Phe | Thr | Ile | Asp | Pro | Ala | | | |
| | | 1060 | | | | 1065 | | | 1070 | | | | | | | | | | |
| | Trp | Ile | Arg | Pro | Gly | Tyr | Pro | Val | Ile | Val | Trp | Thr | Tyr | Trp | Thr | Pro | | | |
| | 1075 | | | | 1080 | | | | 1085 | | | | | | | | | | |
| 30 | Asp | Gln | Arg | Arg | Ile | Ser | Val | Gln | Leu | Gly | His | Asp | Thr | Ile | Met | Gly | | | |
| | 1090 | | | | 1095 | | | | 1100 | | | | | | | | | | |
| | Met | Cys | Lys | Val | Gln | Lys | Glu | Thr | Cys | Gln | Met | Thr | Ser | Ser | Arg | Pro | | | |
| | 1105 | | | | 1110 | | | | 1115 | | | 1120 | | | | | | | |
| | Val | Leu | Gly | Cys | Val | Arg | Ser | Thr | Thr | Gly | Leu | Gly | Phe | Ile | His | Thr | | | |
| 35 | | | 1125 | | | | 1130 | | | | 1135 | | | | | | | | |
| | Ala | Leu | Met | Gly | Ile | Tyr | Ile | Gly | Thr | Pro | Thr | Tyr | Leu | Leu | Ser | Pro | | | |
| | | 1140 | | | | 1145 | | | 1150 | | | | | | | | | | |
| | Val | Glu | Phe | Ala | Ala | Asn | Pro | Met | Ser | Leu | Phe | Val | Thr | Leu | Ser | Arg | | | |
| | 1155 | | | | 1160 | | | | 1165 | | | | | | | | | | |
| 40 | Tyr | Lys | Ile | Lys | Asp | Thr | Tyr | Ala | Thr | Pro | Gln | Met | Leu | Asp | His | Ala | | | |

| | | |
|---|------|------|
| 1170 | 1175 | 1180 |
| Met Asn Ser Met Gln Ala Lys Gly Phe Thr Leu His Glu Leu Lys Asn | | |
| 1185 | 1190 | 1195 |
| Met Met Ile Thr Ala Glu Ser Arg Pro Arg Val Asp Val Phe Gln Lys | | 1200 |
| 5 | 1205 | 1210 |
| Val Arg Leu His Phe Ala Gly Ala Gly Leu Asp Arg Thr Ala Ile Asn | | 1215 |
| | 1220 | 1225 |
| Thr Val Tyr Ser His Val Leu Asn Pro Met Val Ala Ser Arg Ser Tyr | | 1230 |
| 1235 | 1240 | 1245 |
| 10Met Cys Ile Glu Pro Ile Glu Leu Trp Leu Asp Thr Gln Ala Leu Arg | | |
| 1250 | 1255 | 1260 |
| Arg Gly Leu Val Ile Pro Val Asp Pro Glu Ser Asp Pro Leu Ala Leu | | |
| 1265 | 1270 | 1275 |
| Leu Val Gln Asp Ser Gly Met Val Pro Val Ser Thr Gln Ile Ala Ile | | 1280 |
| 15 | 1285 | 1290 |
| Ile Asn Pro Glu Ser Arg Ile His Cys Leu Asp Gly Glu Tyr Gly Glu | | 1295 |
| | 1300 | 1305 |
| Ile Trp Val Asp Ser Glu Ala Cys Val Lys Ser Phe Tyr Gly Ser Lys | | 1310 |
| 1315 | 1320 | 1325 |
| 20Asp Ala Phe Asp Ala Glu Arg Phe Asp Gly Arg Ala Leu Asp Gly Asp | | |
| 1330 | 1335 | 1340 |
| Pro Asn Ile Gln Tyr Ile Arg Thr Gly Asp Leu Gly Phe Leu His Asn | | |
| 1345 | 1350 | 1355 |
| Val Ser Arg Pro Ile Gly Pro Asn Gly Ala Gln Val Asp Met Gln Val | | 1360 |
| 25 | 1365 | 1370 |
| Leu Phe Val Leu Gly Asn Ile Gly Glu Thr Phe Glu Ile Asn Gly Leu | | 1375 |
| | 1380 | 1385 |
| Ser His Phe Pro Met Asp Ile Glu Asn Ser Val Glu Lys Cys His Arg | | 1390 |
| 1395 | 1400 | 1405 |
| 30Asn Ile Val Ala Asn Gly Cys Ala Val Phe Gln Ala Gly Gly Leu Val | | |
| 1410 | 1415 | 1420 |
| Val Val Leu Val Glu Val Asn Arg Lys Pro Tyr Leu Ala Ser Ile Val | | |
| 1425 | 1430 | 1435 |
| Pro Val Ile Val Asn Ala Ile Leu Asn Glu His Gln Ile Ile Val Asp | | 1440 |
| 35 | 1445 | 1450 |
| Ile Val Ala Phe Val Asn Lys Gly Asp Phe Pro Arg Ser Arg Leu Gly | | 1455 |
| | 1460 | 1465 |
| Glu Lys Gln Arg Gly Lys Ile Leu Gly Gly Trp Val Ser Arg Lys Leu | | 1470 |
| 1475 | 1480 | 1485 |
| 40Arg Thr Leu Ala Gln Phe Ser Ile Arg Asp Met Asp Ala Glu Ser Thr | | |

28

| | | |
|---|------|------|
| 1490 | 1495 | 1500 |
| Ala Gly Asp Met Met Asp Pro Ser Arg Ala Ser Met Val Ser Val Arg | | |
| 1505 | 1510 | 1515 |
| Ser Gly Gly Gly Ala Ala Pro Gly Ser Ser Ser Leu Arg Asn Val Glu | | |
| 5 | 1525 | 1530 |
| Pro Ala Pro Gln Ile Leu Glu Glu Glu His Asp Gln Met Thr Pro Arg | | |
| | 1540 | 1545 |
| His Glu Tyr Glu Ala Ala Pro Thr Met Ile Ser Glu Leu Pro Asp Gly | | |
| | 1555 | 1560 |
| 10Gln Glu Thr Pro Thr Gly Phe Gln His Ser Gln Tyr Glu His Pro Pro | | |
| | 1570 | 1575 |
| Gln Ser Ala Gly Ser Gln Ala Pro Ala Gln Leu Asn Leu Ser His Gln | | |
| 1585 | 1590 | 1595 |
| Pro Asp Gln Gly Phe Asp Met Asp Phe Ser Arg Tyr Ser Ser Ala Glu | | |
| 15 | 1605 | 1610 |
| Pro Asp His Gly Pro Val His Arg Arg Pro Val Pro Gly Gln Ala Gln | | |
| | 1620 | 1625 |
| Gln Pro Glu Pro Met Gln Gly Tyr Gly Gln Ala Pro Pro Gln Ile Arg | | |
| | 1635 | 1640 |
| 20Leu Pro Gly Val Asp Gly Arg Glu Glu Gly Gly Phe Trp Ser Gln Gln | | |
| | 1650 | 1655 |
| Glu Lys Asn Glu Lys Ser Glu Glu Asp Trp Thr Thr Asp Ala Met Met | | |
| 1665 | 1670 | 1675 |
| His Met Asn Leu Ala Gly Asp Met Lys Pro Pro Arg | | |
| 25 | 1685 | 1690 |

<210> 42

<211> 2369

<212> DNA

30<213> *Alternaria solani*

<400> 42

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| 35actatgagag tcggtgcttt tggctaccg ataccagatg cgacgctggc cgtcgtcgat | 180 |
| ccggaaacta atcttttgtg ttcaccctat tccataggag agatctgggt agactcgcca | 240 |
| tcattgtccg gagggttttg gcagctgcag aagcacactg agactatttt ccacgctcgg | 300 |
| ccatatcggt tcgtagaggg cagcccaacc ccgcaactac tcgaactgga gtttctacgc | 360 |
| actggactgc tcggatgcgt ggtagaaggc aaaatcttcg tattaggcct gtacgaggac | 420 |
| 40cggattaggc agcgcgttga atgggtagag cacggtcagc tagaagccga acataggtat | 480 |

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```

<210> 43

35<211> 758

<212> PRT

<213> *Alternaria solani*

<400> 43

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30

| | | | |
|---|-----|-----|-----|
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| Ala Leu Lys Leu Asn Glu Val Val Val Leu Ala Ile Gly Glu Glu Val | | | |
| | 20 | 25 | 30 |
| Ser Lys Arg Val Asn Glu Pro Gly Thr Met Arg Val Gly Ala Phe Gly | | | |
| 5 | 35 | 40 | 45 |
| Tyr Pro Ile Pro Asp Ala Thr Leu Ala Val Val Asp Pro Glu Thr Asn | | | |
| | 50 | 55 | 60 |
| Leu Leu Cys Ser Pro Tyr Ser Ile Gly Glu Ile Trp Val Asp Ser Pro | | | |
| 65 | 70 | 75 | 80 |
| 10Ser Leu Ser Gly Gly Phe Trp Gln Leu Gln Lys His Thr Glu Thr Ile | | | |
| | 85 | 90 | 95 |
| Phe His Ala Arg Pro Tyr Arg Phe Val Glu Gly Ser Pro Thr Pro Gln | | | |
| | 100 | 105 | 110 |
| Leu Leu Glu Leu Glu Phe Leu Arg Thr Gly Leu Leu Gly Cys Val Val | | | |
| 15 | 115 | 120 | 125 |
| Glu Gly Lys Ile Phe Val Leu Gly Leu Tyr Glu Asp Arg Ile Arg Gln | | | |
| | 130 | 135 | 140 |
| Arg Val Glu Trp Val Glu His Gly Gln Leu Glu Ala Glu His Arg Tyr | | | |
| 145 | 150 | 155 | 160 |
| 20Phe Phe Val Gln His Leu Val Thr Ser Ile Met Lys Ala Val Pro Lys | | | |
| | 165 | 170 | 175 |
| Ile Tyr Asp Cys Ser Ser Phe Asp Ser Tyr Val Asn Gly Glu Tyr Leu | | | |
| | 180 | 185 | 190 |
| Pro Ile Ile Leu Ile Glu Thr Gln Ala Ala Ser Thr Ala Pro Thr Asn | | | |
| 25 | 195 | 200 | 205 |
| Pro Gly Gly Pro Pro Gln Gln Leu Asp Ile Pro Phe Leu Asp Ser Leu | | | |
| | 210 | 215 | 220 |
| Ser Glu Arg Cys Met Glu Val Leu Tyr Gln Glu His His Leu Arg Val | | | |
| 225 | 230 | 235 | 240 |
| 30Tyr Cys Val Met Ile Thr Ala Pro Asn Thr Leu Pro Arg Val Ile Lys | | | |
| | 245 | 250 | 255 |
| Asn Gly Arg Arg Glu Ile Gly Asn Met Leu Cys Arg Arg Glu Phe Asp | | | |
| | 260 | 265 | 270 |
| Asn Gly Ser Leu Pro Cys Val His Val Lys Phe Gly Val Glu Arg Ser | | | |
| 35 | 275 | 280 | 285 |
| Val Gln Asn Ile Ala Leu Gly Asp Asp Pro Ala Gly Gly Met Trp Ser | | | |
| | 290 | 295 | 300 |
| Tyr Glu Ala Ser Met Ala Arg Gln Gln Phe Leu Met Leu Gln Asp Lys | | | |
| 305 | 310 | 315 | 320 |
| 40Gln Tyr Ser Gly Val Asp His Arg Glu Val Val Ile Asp Asp Arg Thr | | | |

31

| | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | | 325 | | | | | 330 | | | | 335 | | | |
| | Ser | Thr | Pro | Leu | Asn | Gln | Phe | Ser | Asn | Ile | His | Asp | Leu | Met | Gln | Trp |
| | | | | 340 | | | | | 345 | | | | 350 | | | |
| | Arg | Val | Gln | Arg | Gln | Ala | Glu | Glu | Leu | Ala | Tyr | Cys | Thr | Val | Asp | Gly |
| 5 | | | 355 | | | | | 360 | | | | | 365 | | | |
| | Arg | Gly | Lys | Glu | Gly | Lys | Gly | Val | Asn | Trp | Lys | Lys | Phe | Asp | Gln | Lys |
| | | | 370 | | | | 375 | | | | | 380 | | | | |
| | Val | Ala | Gly | Val | Ala | Met | Tyr | Leu | Lys | Asn | Lys | Val | Lys | Gly | Gln | Thr |
| | 385 | | | | 390 | | | | | 395 | | | | | 400 | |
| 10 | Gly | Asp | His | Leu | Leu | Leu | Met | Tyr | Thr | His | Ser | Glu | Asp | Phe | Val | Tyr |
| | | | | 405 | | | | | 410 | | | | 415 | | | |
| | Ala | Val | His | Ala | Cys | Phe | Val | Leu | Gly | Ala | Val | Cys | Ile | Pro | Met | Ala |
| | | | | 420 | | | | 425 | | | | 430 | | | | |
| | Pro | Ile | Asp | Gln | Asn | Arg | Leu | Asn | Glu | Asp | Ala | Pro | Ala | Leu | Leu | His |
| 15 | | | 435 | | | | 440 | | | | | 445 | | | | |
| | Ile | Ile | Ala | Asp | Phe | Lys | Val | Lys | Ala | Ile | Leu | Val | Asn | Ala | Gly | Val |
| | | | 450 | | | | 455 | | | | 460 | | | | | |
| | Asp | His | Leu | Met | Lys | Val | Lys | Gln | Val | Ser | Gln | His | Ile | Lys | Gln | Ser |
| | 465 | | | | 470 | | | | 475 | | | | 480 | | | |
| 20 | Ala | Val | Ile | Leu | Lys | Ile | Asn | Val | Pro | Asn | Thr | Tyr | Asn | Thr | Thr | Lys |
| | | | | 485 | | | | 490 | | | | 495 | | | | |
| | Pro | Pro | Lys | Gln | Ser | Ser | Gly | Cys | Arg | Asp | Leu | Lys | Leu | Thr | Ile | Arg |
| | | | 500 | | | | 505 | | | | 510 | | | | | |
| | Pro | Ala | Trp | Ile | Gln | Ser | Gly | Phe | Pro | Val | Leu | Val | Trp | Thr | Tyr | Trp |
| 25 | | | 515 | | | | 520 | | | | 525 | | | | | |
| | Thr | Pro | Asp | Gln | Arg | Arg | Ile | Ala | Val | Gln | Leu | Gly | His | Ser | Gln | Ile |
| | | | 530 | | | | 535 | | | | 540 | | | | | |
| | Met | Ala | Leu | Cys | Lys | Val | Gln | Lys | Glu | Thr | Cys | Gln | Met | Thr | Ser | Thr |
| | 545 | | | | 550 | | | | 555 | | | | 560 | | | |
| 30 | Arg | Pro | Val | Leu | Gly | Cys | Val | Arg | Ser | Thr | Ile | Gly | Leu | Gly | Phe | Ile |
| | | | | 565 | | | | 570 | | | | 575 | | | | |
| | His | Thr | Cys | Val | Met | Gly | Ile | Phe | Leu | Ala | Ala | Pro | Thr | Tyr | Leu | Val |
| | | | 580 | | | | 585 | | | | 590 | | | | | |
| | Ser | Pro | Val | Asp | Phe | Ala | Gln | Asn | Pro | Asn | Ile | Leu | Phe | Gln | Thr | Met |
| 35 | | | 595 | | | | 600 | | | | 605 | | | | | |
| | Ser | Arg | Tyr | Lys | Ile | Lys | Asp | Ala | Tyr | Ala | Thr | Ser | Gln | Met | Leu | Asp |
| | | | 610 | | | | 615 | | | | 620 | | | | | |
| | His | Ala | Ile | Ala | Arg | Gly | Ala | Gly | Lys | Asn | Met | Ala | Leu | His | Glu | Leu |
| | 625 | | | | 630 | | | | 635 | | | 640 | | | | |
| 40 | Lys | Asn | Leu | Met | Ile | Ala | Thr | Asp | Gly | Arg | Pro | Arg | Val | Asp | Val | Tyr |

32

| | | | |
|----|---|-----|-----|
| | 645 | 650 | 655 |
| | Gln Arg Val Arg Val His Phe Ser Pro Ala Ser Leu Asp Arg Thr Ala | | |
| | 660 | 665 | 670 |
| | Ile Asn Thr Val Tyr Ser His Val Leu Asn Pro Met Val Ala Ser Arg | | |
| 5 | 675 | 680 | 685 |
| | Ser Tyr Met Cys Ile Glu Pro Ile Glu Leu His Leu Asp Val Gly Ala | | |
| | 690 | 695 | 700 |
| | Leu Arg Arg Gly Leu Ile Met Pro Val Asp Pro Asp Thr Glu Pro Gly | | |
| | 705 | 710 | 715 |
| 10 | Ala Leu Leu Val Gln Asp Ser Gly Met Val Pro Val Ser Thr Gln Ile | | |
| | 725 | 730 | 735 |
| | Ser Ile Val Asn Pro Glu Thr Asn Gln Leu Cys Leu Val Gly Glu Tyr | | |
| | 740 | 745 | 750 |
| | Gly Glu Ile Trp Val Gln | | |
| 15 | 755 | | |

<210> 44

<211> 2320

<212> DNA

20<213> Pyrenophora teres

<400> 44

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| 25acaatgcgag | ttggcgcttt | tggataccca | ataccagatg | cgacgctagc | cgtcgtagat | 180 |
| ccagagacga | atctcttgtg | ttcaccctac | tcgataggag | agatttgggt | agactcacct | 240 |
| tcattgtctg | gtgggttctg | gcaattgcag | aagcacactg | aaactatatt | tcacgcccgc | 300 |
| ccataccgct | ttgtggaggg | cagtcctacc | ccgcagttgc | ttgagcttga | gtttctccgg | 360 |
| acaggcttac | tcggattcgt | cgtagagggc | aagggtctta | tccttggtct | ctatgaagat | 420 |
| 30cgcatcaggc | agcgcgttga | atgggtagaa | catggctcagc | tggaagctga | acacagatac | 480 |
| ttcttcgtgc | agcacctcgt | caccagtatc | atgaaggctg | ttcccaagat | ctacgactgg | 540 |
| taagtcttct | catgttttag | atgagcgttc | taacactatg | cagctcatct | ttcgactcgt | 600 |
| acgtcaatgg | cgaatacctg | cctatcatcc | tcatcgagac | acaggctgca | tcgacagccc | 660 |
| ctacgaaccc | tggtggaccg | ccacagcaac | tcgacatccc | cttcctagac | tcactgtctg | 720 |
| 35agcgatgcat | ggaagtgttg | tatcaagaac | accatctgcg | agtatactgc | gtcatgatca | 780 |
| cagcgccaaa | cacattacca | cgagttgtta | agaatggctg | acgagaaatt | ggcaacatgc | 840 |
| tctgtcgaag | agaatttgat | aatggctcat | taccttgtgt | ccacgtcaag | tttgggtgtg | 900 |
| agaggctcag | tctcaacatc | gcgttgggtg | atgacccctc | cggaggcatg | tggtcatatg | 960 |
| aagcctcgat | ggcgcgtcag | cagttcttga | tgctccaaga | caagcagtat | tctggagtag | 1020 |
| 40atcaccgcga | agtcgtcatg | gatgacagaa | catcgacacc | tctcaaccaa | ttctccaaca | 1080 |


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<210> 45

<211> 758

25<212> PRT

<213> *Pyrenophora teres*

<400> 45

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          20          25          30
Ser Lys Arg Ala Asn Glu Pro Gly Thr Met Arg Val Gly Ala Phe Gly
          35          40          45
35Tyr Pro Ile Pro Asp Ala Thr Leu Ala Val Val Asp Pro Glu Thr Asn
          50          55          60
Leu Leu Cys Ser Pro Tyr Ser Ile Gly Glu Ile Trp Val Asp Ser Pro
65          70          75          80
Ser Leu Ser Gly Gly Phe Trp Gln Leu Gln Lys His Thr Glu Thr Ile
40          85          90          95

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34

Phe His Ala Arg Pro Tyr Arg Phe Val Glu Gly Ser Pro Thr Pro Gln
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 115 120 125
 5Glu Gly Lys Val Phe Ile Leu Gly Leu Tyr Glu Asp Arg Ile Arg Gln
 130 135 140
 Arg Val Glu Trp Val Glu His Gly Gln Leu Glu Ala Glu His Arg Tyr
 145 150 155 160
 Phe Phe Val Gln His Leu Val Thr Ser Ile Met Lys Ala Val Pro Lys
 10 165 170 175
 Ile Tyr Asp Cys Ser Ser Phe Asp Ser Tyr Val Asn Gly Glu Tyr Leu
 180 185 190
 Pro Ile Ile Leu Ile Glu Thr Gln Ala Ala Ser Thr Ala Pro Thr Asn
 195 200 205
 15Pro Gly Gly Pro Pro Gln Gln Leu Asp Ile Pro Phe Leu Asp Ser Leu
 210 215 220
 Ser Glu Arg Cys Met Glu Val Leu Tyr Gln Glu His His Leu Arg Val
 225 230 235 240
 Tyr Cys Val Met Ile Thr Ala Pro Asn Thr Leu Pro Arg Val Val Lys
 20 245 250 255
 Asn Gly Arg Arg Glu Ile Gly Asn Met Leu Cys Arg Arg Glu Phe Asp
 260 265 270
 Asn Gly Ser Leu Pro Cys Val His Val Lys Phe Gly Val Glu Arg Ser
 275 280 285
 25Val Leu Asn Ile Ala Leu Gly Asp Asp Pro Ser Gly Gly Met Trp Ser
 290 295 300
 Tyr Glu Ala Ser Met Ala Arg Gln Gln Phe Leu Met Leu Gln Asp Lys
 305 310 315 320
 Gln Tyr Ser Gly Val Asp His Arg Glu Val Val Met Asp Asp Arg Thr
 30 325 330 335
 Ser Thr Pro Leu Asn Gln Phe Ser Asn Ile His Asp Leu Met Gln Trp
 340 345 350
 Arg Val Ser Arg Gln Ala Glu Glu Leu Ala Tyr Cys Thr Val Asp Gly
 355 360 365
 35Arg Gly Lys Glu Gly Lys Gly Val Asn Trp Lys Lys Phe Asp Gln Lys
 370 375 380
 Val Ala Gly Val Ala Met Tyr Leu Lys Asn Lys Val Lys Val Gln Thr
 385 390 395 400
 Gly Asp His Leu Leu Leu Met Tyr Thr His Ser Glu Asp Phe Val Tyr
 40 405 410 415

35

Ala Val His Ala Cys Phe Val Leu Gly Ala Val Cys Ile Pro Met Ala
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 Pro Ile Asp Gln Asn Arg Leu Asn Glu Asp Ala Pro Ala Leu Leu His
 435 440 445
 5Ile Leu Ala Asp Phe Lys Val Lys Ala Ile Leu Val Asn Ala Asp Val
 450 455 460
 Asp His Leu Met Lys Val Lys Gln Val Ser Gln His Ile Lys Gln Ser
 465 470 475 480
 Ala Ala Ile Phe Lys Ile Asn Val Pro His Thr Tyr Asn Thr Thr Lys
 10 485 490 495
 Pro Pro Lys Gln Ser Ser Gly Cys Arg Asp Leu Lys Leu Thr Ile Arg
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 Pro Ala Trp Val Gln Pro Gly Phe Pro Val Leu Val Trp Thr Tyr Trp
 515 520 525
 15Thr Pro Asp Gln Arg Arg Ile Ala Val Gln Leu Gly His Ser Gln Ile
 530 535 540
 Met Ala Leu Gly Lys Val Gln Lys Glu Thr Cys Gln Met Thr Ser Thr
 545 550 555 560
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 His Thr Cys Ile Met Gly Ile Phe Leu Ala Ala Pro Thr Tyr Leu Val
 580 585 590
 Ser Pro Val Asp Phe Ala Gln Asn Pro Asn Ile Leu Phe Gln Thr Leu
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 25Ser Arg Tyr Lys Ile Lys Asn Ala Tyr Ala Thr Ser Gln Met Leu Asp
 610 615 620
 His Ala Ile Ala Arg Gly Ala Gly Lys Asn Met Ala Leu His Glu Leu
 625 630 635 640
 Lys Asn Leu Met Ile Ala Thr Asp Gly Arg Pro Arg Val Asp Val Tyr
 30 645 650 655
 Gln Arg Val Arg Val His Phe Ser Pro Ala Ser Leu Asp Arg Thr Ala
 660 665 670
 Ile Asn Thr Val Tyr Ser His Val Leu Asn Pro Met Val Ala Ser Arg
 675 680 685
 35Ser Tyr Met Cys Ile Glu Pro Ile Glu Leu His Leu Asp Val Asn Ala
 690 695 700
 Leu Arg Arg Gly Leu Ile Met Pro Val Asp Pro Asp Thr Glu Pro Gly
 705 710 715 720
 Ala Leu Met Val Gln Asp Ser Gly Met Val Pro Val Ser Thr Gln Ile
 40 725 730 735

36

Ala Ile Val Asn Pro Glu Thr Asn Gln Leu Cys Leu Val Gly Glu Tyr

740

745

750

Gly Glu Ile Trp Val Gln

755

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<210> 46

<211> 2435

<212> DNA

<213> *Coccidioides immitis*

10

<400> 46

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37

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<210> 47

15<211> 812

<212> PRT

<213> *Coccidioides immitis*

<400> 47

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Glu Phe Ala Arg Ala Pro Thr Gly Asp Leu Arg Gly Val Val Met Ser
25           35           40           45
His Arg Thr Ile Met His Gln Met Cys Cys Met Ser Ala Ile Val Ser
           50           55           60
Thr Ile Pro Thr Asp Ser Asn Asn Ser Gly Lys Pro Val Pro Arg Pro
65           70           75           80
30His Gly Glu Ile Leu Met Ser Tyr Leu Asp Pro Arg Gln Gly Ile Gly
           85           90           95
Met Ile Leu Gly Val Leu Leu Thr Val Tyr Ala Gly Asn Thr Thr Val
           100          105          110
Trp Leu Glu Ser Leu Ala Val Glu Thr Pro Gly Leu Tyr Ala Ser Leu
35           115          120          125
Ile Thr Lys Tyr Arg Ala Ala Leu Leu Ala Ala Asp Tyr Pro Gly Leu
           130          135          140
Lys Arg Ala Val Tyr Asn Tyr Gln Gln Asp Pro Met Ala Thr Arg Asn
145           150           155           160
40Phe Lys Lys Asn Ser Glu Pro Asn Phe Ser Ser Leu Lys Leu Cys Leu

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38

| | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | | 165 | | | | | 170 | | | | | 175 | | |
| | Ile | Asp | Thr | Leu | Thr | Val | Asp | Cys | Glu | Phe | His | Glu | Ile | Leu | Ala | Asp |
| | | | | 180 | | | | | 185 | | | | | 190 | | |
| | Arg | Trp | Leu | Arg | Pro | Leu | Arg | Asn | Pro | Arg | Ala | Arg | Glu | Leu | Val | Thr |
| 5 | | | 195 | | | | | 200 | | | | | 205 | | | |
| | Pro | Met | Leu | Cys | Leu | Pro | Glu | His | Gly | Gly | Met | Val | Ile | Ser | Leu | Arg |
| | | | 210 | | | | | 215 | | | | | 220 | | | |
| | Asp | Trp | Leu | Gly | Gly | Glu | Glu | Arg | Met | Gly | Cys | Pro | Leu | Lys | His | Glu |
| | 225 | | | | | 230 | | | | 235 | | | | | 240 | |
| 10 | Val | Leu | Pro | Pro | Glu | Lys | Gln | Lys | Asp | Lys | Ser | Glu | Gly | Glu | Lys | Lys |
| | | | | | | 245 | | | | 250 | | | | | 255 | |
| | Glu | Glu | Glu | Lys | Gly | Gly | Glu | Pro | Lys | Ala | Thr | Phe | Gly | Ser | Ser | Leu |
| | | | | 260 | | | | | 265 | | | | | 270 | | |
| | Ile | Gly | Gly | Ser | Ala | Ala | Pro | Ile | Arg | Lys | Glu | Gly | Pro | Arg | Asn | Asp |
| 15 | | | 275 | | | | | 280 | | | | | | 285 | | |
| | Leu | Gly | Glu | Val | Leu | Leu | Asp | Lys | Glu | Ala | Leu | Lys | Asn | Asn | Glu | Ile |
| | | | 290 | | | | | 295 | | | | | 300 | | | |
| | Val | Ile | Leu | Ala | Ile | Gly | Glu | Glu | Ala | Arg | Arg | Leu | Ala | Asp | Thr | Thr |
| | 305 | | | | | 310 | | | | 315 | | | | | 320 | |
| 20 | Pro | Asn | Ala | Val | Arg | Val | Gly | Ala | Phe | Gly | Tyr | Pro | Ile | Pro | Asp | Ala |
| | | | | | | 325 | | | | 330 | | | | | 335 | |
| | Thr | Leu | Ala | Ile | Val | Asp | Pro | Glu | Thr | Gly | Leu | Leu | Cys | Thr | Pro | Asn |
| | | | | 340 | | | | | 345 | | | | | 350 | | |
| | Val | Val | Gly | Glu | Ile | Trp | Val | Asp | Ser | Pro | Ser | Leu | Ser | Gly | Gly | Phe |
| 25 | | | 355 | | | | | 360 | | | | | | 365 | | |
| | Trp | Ala | Leu | Pro | Lys | Gln | Thr | Glu | Ser | Ile | Phe | His | Ala | Arg | Pro | Tyr |
| | | | 370 | | | | 375 | | | | 380 | | | | | |
| | Arg | Phe | Gln | Gly | Gly | Gly | Pro | Thr | Pro | Val | Ile | Val | Glu | Pro | Glu | Phe |
| | 385 | | | | | 390 | | | | | 395 | | | | 400 | |
| 30 | Leu | Arg | Thr | Gly | Leu | Leu | Gly | Cys | Val | Ile | Glu | Gly | Gln | Ile | Phe | Val |
| | | | | 405 | | | | | | 410 | | | | | 415 | |
| | Leu | Gly | Leu | Tyr | Glu | Asp | Arg | Leu | Arg | Gln | Lys | Val | Glu | Trp | Val | Glu |
| | | | | 420 | | | | | 425 | | | | | 430 | | |
| | His | Gly | Val | Glu | Val | Ala | Glu | His | Arg | Tyr | Phe | Phe | Val | Gln | His | Leu |
| 35 | | | 435 | | | | | 440 | | | | | | 445 | | |
| | Ile | Leu | Ser | Ile | Met | Lys | Asn | Val | Pro | Lys | Ile | His | Asp | Cys | Ser | Ala |
| | | | 450 | | | | | 455 | | | | | 460 | | | |
| | Phe | Asp | Val | Phe | Val | Asn | Glu | Glu | His | Leu | Pro | Val | Val | Val | Leu | Glu |
| | 465 | | | | | 470 | | | | 475 | | | | | 480 | |
| 40 | Ser | Tyr | Thr | Ala | Ser | Thr | Ala | Pro | Val | Ala | Ser | Gly | Gln | Ser | Pro | Arg |

39

| | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | | 485 | | | | | 490 | | | | | 495 | | |
| | Gln | Leu | Asp | Val | Pro | Leu | Leu | Asp | Ser | Leu | Ala | Glu | Lys | Cys | Met | Gly |
| | | | | 500 | | | | | 505 | | | | | 510 | | |
| | Val | Leu | Tyr | Gln | Glu | His | His | Leu | Arg | Val | Tyr | Cys | Val | Met | Ile | Thr |
| 5 | | | 515 | | | | | 520 | | | | | 525 | | | |
| | Ala | Pro | Asn | Thr | Leu | Pro | Arg | Val | Leu | Lys | Asn | Gly | Arg | Gln | Glu | Ile |
| | | | 530 | | | | 535 | | | | | 540 | | | | |
| | Gly | Asn | Met | Leu | Cys | Arg | Lys | Glu | Phe | Asp | Asn | Gly | Ser | Leu | Pro | Cys |
| | 545 | | | | | 550 | | | | 555 | | | | | 560 | |
| 10 | Glu | His | Val | Lys | Phe | Ser | Val | Glu | Arg | Ser | Val | Leu | Ser | Leu | Pro | Ile |
| | | | | | | 565 | | | | 570 | | | | 575 | | |
| | Gly | Val | Asp | Pro | Val | Gly | Gly | Ile | Trp | Ser | Val | Pro | Ser | Ser | Ala | Ala |
| | | | | 580 | | | | | 585 | | | | | 590 | | |
| | Arg | Gln | Asp | Ala | Leu | Ala | Met | Gln | Glu | Lys | Gln | Tyr | Ser | Gly | Val | Asp |
| 15 | | | 595 | | | | | 600 | | | | | 605 | | | |
| | Leu | Arg | Asp | Val | Ile | Met | Asp | Asp | Arg | Thr | Ser | Thr | Pro | Leu | Asn | Asn |
| | | | 610 | | | | 615 | | | | | 620 | | | | |
| | Phe | Asn | Ser | Ile | Val | Asp | Leu | Leu | Gln | Trp | Arg | Val | Ser | Arg | Gln | Gly |
| | 625 | | | | | 630 | | | | 635 | | | | | 640 | |
| 20 | Glu | Glu | Leu | Cys | Tyr | Cys | Ser | Ile | Asp | Gly | Arg | Gly | Arg | Glu | Gly | Lys |
| | | | | | | 645 | | | | 650 | | | | 655 | | |
| | Gly | Ile | Thr | Trp | Lys | Lys | Phe | Asp | Ser | Lys | Val | Ala | Ala | Val | Ala | Ala |
| | | | | 660 | | | | 665 | | | | | 670 | | | |
| | Tyr | Leu | Lys | Asn | Lys | Val | Lys | Leu | Arg | Pro | Gly | Asp | His | Val | Ile | Leu |
| 25 | | | 675 | | | | | 680 | | | | | 685 | | | |
| | Met | Tyr | Thr | His | Ser | Glu | Glu | Tyr | Val | Phe | Ala | Val | His | Ala | Cys | Phe |
| | | | 690 | | | | 695 | | | | 700 | | | | | |
| | Cys | Leu | Gly | Leu | Val | Ala | Ile | Pro | Ile | Ser | Pro | Val | Asp | Gln | Asn | Arg |
| | 705 | | | | | 710 | | | | 715 | | | | | 720 | |
| 30 | Leu | Ser | Glu | Asp | Ala | Pro | Ala | Leu | Leu | His | Val | Ile | Val | Asp | Phe | Arg |
| | | | | | | 725 | | | | 730 | | | | 735 | | |
| | Val | Lys | Ala | Ile | Leu | Val | Asn | Gly | Glu | Val | Asn | Asp | Leu | Leu | Lys | Gln |
| | | | | 740 | | | | 745 | | | | | 750 | | | |
| | Lys | Ile | Val | Ser | Gln | His | Ile | Lys | Gln | Ser | Ala | His | Val | Val | Arg | Thr |
| 35 | | | 755 | | | | | 760 | | | | | 765 | | | |
| | Ser | Val | Pro | Ser | Val | Tyr | Asn | Thr | Ser | Lys | Pro | Pro | Lys | Gln | Ser | His |
| | | | 770 | | | | 775 | | | | | 780 | | | | |
| | Gly | Cys | Arg | His | Leu | Gly | Phe | Thr | Met | Asn | Pro | Gln | Trp | Leu | Asn | Ser |
| | 785 | | | | 790 | | | | | 795 | | | | | 800 | |
| 40 | Lys | Gln | Pro | Ala | Val | Ile | Trp | Thr | Tyr | Trp | Thr | Pro | | | | |

40

805

810

<210> 48

<211> 1836

5<212> DNA

<213> *Cochliobolus heterostrophus*

<400> 48

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41

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<211> 611

<212> PRT

<213> Cochliobolus heterostrophus

5

<400> 49

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Tyr Ser Tyr Gly Leu Thr Gly Val Asp Gln Val Gly Asn Phe Leu Trp
      35           40           45
Val Asp Thr Phe Leu Tyr Met Leu Ile Gly Ile Ser Gly Met Leu Leu
      50           55           60
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   65           70           75           80
Ala Met Gly Ser Pro Arg Gln Lys Tyr Trp Glu Thr Asn Arg Thr Ser
      85           90           95
Trp Trp Pro Trp Leu Asn Arg His Ile Leu Val Ala Pro Leu Trp Lys
20           100          105          110
Lys Lys His Asn Ala Gln Phe Gln Ile Ser Ser Ala Ile Asp Asn Gly
      115          120          125
Thr Leu Pro Gly Arg Trp His Thr Ile Met Leu Leu Ile Tyr Val Gly
      130          135          140
25Leu Asn Val Ala Trp Cys Leu Ala Leu Pro Tyr Asp Val Leu Asp His
   145          150          155          160
Arg Glu Thr Leu Ala Ala Leu Arg Gly Arg Ser Gly Thr Leu Ala Ala
      165          170          175
Leu Asn Leu Ile Pro Thr Ile Leu Phe Ala Leu Arg Asn Asn Pro Leu
30           180          185          190
Ile Ser Leu Leu Gln Val Ser Tyr Asp Asp Phe Asn Leu Phe His Arg
      195          200          205
Trp Ala Ala Arg Ile Thr Ile Ala Glu Ala Ile Val His Thr Ala Ala
      210          215          220
35Trp Leu Tyr Asn Thr Lys Ala Gly Gly Gly Trp His Ala Val Val Ala
   225          230          235          240
Ala Leu His Thr Glu Gly Ser Tyr Gly Trp Gly Met Gly Gly Thr Val
      245          250          255
Ala Phe Thr Phe Ile Gly Ile Gln Ala Trp Ser Pro Phe Arg His Ala
40           260          265          270

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42

Phe Tyr Glu Thr Phe Leu Asn Ile His Arg Val Met Val Ile Ala Ala
 275 280 285
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 290 295 300
 5Pro Trp Met Tyr Leu Ile Phe Ile Phe Trp Ala Ala Glu Trp Phe Leu
 305 310 315 320
 Arg Leu Cys Ser Ile Cys Tyr Tyr Gly Phe Ser Leu Lys Gln Arg Ser
 325 330 335
 Ser Ile Thr Val Glu Ala Leu Pro Gly Glu Ala Val Arg Leu Thr Ile
 10 340 345 350
 Asn Met Val Arg Glu Trp Thr Pro Arg Pro Gly Cys His Val His Met
 355 360 365
 Trp Met Pro Arg Leu Ser Leu Trp Ser Ser His Pro Phe Ser Val Ala
 370 375 380
 15Trp Ala Ala Thr Leu Thr Asp Asp Ser Lys Glu Met Thr Leu Pro Thr
 385 390 395 400
 Leu Glu Gly Asp Val Thr Met Ile Asn Gly Gln Pro Arg Lys Ser Lys
 405 410 415
 Gln Ile Ser Leu Ile Cys Arg Ala Arg Thr Gly Leu Thr Arg Gln Met
 20 420 425 430
 Tyr Glu Lys Ala Ser Lys Ser Pro Asn Glu Gln Phe Thr Thr Trp Gly
 435 440 445
 Phe Ile Glu Gly Pro Tyr Gly Gly His His Ser Leu Asp Ser Tyr Gly
 450 455 460
 25Thr Cys Val Leu Phe Ala Ala Gly Val Gly Ile Thr His Gln Val Met
 465 470 475 480
 Tyr Leu Lys His Leu Val Asn Gly Phe Asn Asn Gly Thr Thr Ala Thr
 485 490 495
 Gln Lys Ile Val Leu Ile Trp Thr Val Pro Thr Pro Asp Cys Leu Glu
 30 500 505 510
 Trp Val Arg Pro Trp Met Asp Glu Val Leu Arg Met Lys Gly Arg Lys
 515 520 525
 Gln Cys Leu Arg Ile Lys Leu Phe Ile Ser Arg Pro Lys Gly Arg Val
 530 535 540
 35Glu Ser Ser Ser Asp Thr Val Lys Met Tyr Ser Gly Arg Pro Asn Met
 545 550 555 560
 Arg Ser Leu Leu Glu Glu Glu Ala Lys His Arg Val Gly Ala Met Ala
 565 570 575
 Val Thr Val Cys Ala Ser Gly Gly Met Ala Asp Gly Val Arg His Ala
 40 580 585 590

43

Val Arg Pro Leu Leu Thr Glu Gly Ser Val Asp Phe Ile Glu Glu Ala

595

600

605

Phe Thr Tyr

610

5

<210> 50

<211> 6553

<212> DNA

<213> Cochliobolus heterostrophus

10

<400> 50

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| ccgccgcca | caacaccatg | gctgcgacca | accccgagct | gcaggccaaa | ctgcaggagc | 180 |
| 15tggaaccacga | gctcgaggag | ggcgatatta | cacaaaaagg | gtccgtactg | ctgcaccacc | 240 |
| accgccatcc | gcctctctgc | gtgcgcta | cagtcgcata | gctatgaaaa | acgtcgcacc | 300 |
| gtgctgctgt | cgcagtatct | agggcctgac | tttgetgccc | agttgcaggc | cgacctgaac | 360 |
| cagcagaacc | cacccaacc | atccagttag | ggctctcgct | cccgccaccg | atcctttgct | 420 |
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| | gtatggctcg | agacagcgac | catggaaacc | ccgggtctat | atgcacatct | catcaccaaa | 1920 |
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6553

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<211> 530

<212> PRT

<213> Alternaria solani

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| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Ala | Leu | Lys | Leu | Asn | Glu | Val | Val | Val | Leu | Ala | Ile | Gly | Glu | Glu | Val |
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| 15Ser | Lys | Arg | Val | Asn | Glu | Pro | Gly | Thr | Met | Arg | Val | Gly | Ala | Phe | Gly |
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| Tyr | Pro | Ile | Pro | Asp | Ala | Thr | Leu | Ala | Val | Val | Asp | Pro | Glu | Thr | Asn |
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| Leu | Leu | Cys | Ser | Pro | Tyr | Ser | Ile | Gly | Glu | Ile | Trp | Val | Asp | Ser | Pro |
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| Ser | Leu | Ser | Gly | Gly | Phe | Trp | Gln | Leu | Gln | Lys | His | Thr | Glu | Thr | Ile |
| | | | | 85 | | | | | 90 | | | | 95 | | |
| Phe | His | Ala | Arg | Pro | Tyr | Arg | Phe | Val | Glu | Gly | Ser | Pro | Thr | Pro | Gln |
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| Glu | Gly | Lys | Ile | Phe | Val | Leu | Gly | Leu | Tyr | Glu | Asp | Arg | Ile | Arg | Gln |
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| Arg | Val | Glu | Trp | Val | Glu | His | Gly | Gln | Leu | Glu | Ala | Glu | His | Arg | Tyr |
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| Phe | Phe | Val | Gln | His | Leu | Val | Thr | Ser | Ile | Met | Lys | Ala | Val | Pro | Lys |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Ile | Tyr | Asp | Cys | Ser | Ser | Phe | Asp | Ser | Tyr | Val | Asn | Gly | Glu | Tyr | Leu |
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| 35Pro | Ile | Ile | Leu | Ile | Glu | Thr | Gln | Ala | Ala | Ser | Thr | Ala | Pro | Thr | Asn |
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| Pro | Gly | Gly | Pro | Pro | Gln | Gln | Leu | Asp | Ile | Pro | Phe | Leu | Asp | Ser | Leu |
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47

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 Pro Pro Lys Gln Ser Ser Gly Cys Arg Asp Leu Lys Leu Thr Ile Arg
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 Thr Pro
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<213> Pyrenophora teres

5<400> 52

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| Ala | Leu | Lys | Met | Asn | Asp | Val | Val | Val | Leu | Ala | Ile | Gly | Glu | Glu | Ala |
| | | | 20 | | | | | 25 | | | | | 30 | | |
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| | | | 35 | | | | 40 | | | | | 45 | | | |
| Tyr | Pro | Ile | Pro | Asp | Ala | Thr | Leu | Ala | Val | Val | Asp | Pro | Glu | Thr | Asn |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Leu | Leu | Cys | Ser | Pro | Tyr | Ser | Ile | Gly | Glu | Ile | Trp | Val | Asp | Ser | Pro |
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| Ser | Leu | Ser | Gly | Gly | Phe | Trp | Gln | Leu | Gln | Lys | His | Thr | Glu | Thr | Ile |
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| Phe | His | Ala | Arg | Pro | Tyr | Arg | Phe | Val | Glu | Gly | Ser | Pro | Thr | Pro | Gln |
| | | | 100 | | | | | 105 | | | | | 110 | | |
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| | | 115 | | | | | 120 | | | | | 125 | | | |
| Glu | Gly | Lys | Val | Phe | Ile | Leu | Gly | Leu | Tyr | Glu | Asp | Arg | Ile | Arg | Gln |
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| Phe | Phe | Val | Gln | His | Leu | Val | Thr | Ser | Ile | Met | Lys | Ala | Val | Pro | Lys |
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| Pro | Gly | Gly | Pro | Pro | Gln | Gln | Leu | Asp | Ile | Pro | Phe | Leu | Asp | Ser | Leu |
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| Tyr | Cys | Val | Met | Ile | Thr | Ala | Pro | Asn | Thr | Leu | Pro | Arg | Val | Val | Lys |
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49

| | | |
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| Gln Tyr Ser Gly Val Asp His Arg Glu Val Val Met Asp Asp Arg Thr | | |
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| Ser Thr Pro Leu Asn Gln Phe Ser Asn Ile His Asp Leu Met Gln Trp | | |
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| Val Ala Gly Val Ala Met Tyr Leu Lys Asn Lys Val Lys Val Gln Thr | | |
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| 405 | 410 | 415 |
| Ala Val His Ala Cys Phe Val Leu Gly Ala Val Cys Ile Pro Met Ala | | |
| 420 | 425 | 430 |
| 20Pro Ile Asp Gln Asn Arg Leu Asn Glu Asp Ala Pro Ala Leu Leu His | | |
| 435 | 440 | 445 |
| Ile Leu Ala Asp Phe Lys Val Lys Ala Ile Leu Val Asn Ala Asp Val | | |
| 450 | 455 | 460 |
| Asp His Leu Met Lys Val Lys Gln Val Ser Gln His Ile Lys Gln Ser | | |
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| Ala Ala Ile Phe Lys Ile Asn Val Pro His Thr Tyr Asn Thr Thr Lys | | |
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| Pro Pro Lys Gln Ser Ser Gly Cys Arg Asp Leu Lys Leu Thr Ile Arg | | |
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40<400> 53

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 Gly Leu Leu Ala Ser Pro His Ser Val Gly Glu Ile Trp Val Asp Ser
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 Thr Ser Gly Gly Pro Pro Arg Gln Pro Asp Thr Ala Leu Leu Glu Ser
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 Leu Ala Glu Arg Cys Met Glu Val Leu Met Ser Glu His His Leu Arg
 30225 230 235 240
 Leu Tyr Cys Val Met Ile Thr Ala Pro Asp Thr Leu Pro Arg Val Val
 245 250 255
 Lys Asn Gly Arg Arg Glu Ile Gly Asn Met Leu Cys Arg Arg Glu Phe
 260 265 270
 35Asp Leu Gly Asn Leu Pro Cys Val His Val Lys Phe Gly Val Glu His
 275 280 285
 Ala Val Leu Asn Leu Pro Ile Gly Val Asp Pro Ile Gly Gly Ile Trp
 290 295 300
 Ser Pro Leu Ala Ser Asp Ser Arg Ala Glu Phe Leu Leu Pro Ala Asp
 40305 310 315 320

51

| | | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Lys | Gln | Tyr | Ser | Gly | Val | Asp | Arg | Arg | Glu | Val | Val | Ile | Asp | Asp | Arg | |
| | | | | 325 | | | | 330 | | | | 335 | | | | |
| Thr | Ser | Thr | Pro | Leu | Asn | Asn | Phe | Ser | Cys | Ile | Ser | Asp | Leu | Ile | Gln | |
| | | | | 340 | | | | 345 | | | | 350 | | | | |
| 5Trp | Arg | Val | Ala | Arg | Gln | Pro | Glu | Glu | Leu | Ala | Tyr | Cys | Thr | Ile | Asp | |
| | | | | 355 | | | | 360 | | | | 365 | | | | |
| Gly | Lys | Ser | Arg | Glu | Gly | Lys | Gly | Val | Thr | Trp | Lys | Lys | Phe | Asp | Thr | |
| | | | | 370 | | | | 375 | | | | 380 | | | | |
| Lys | Val | Ala | Ser | Val | Ala | Met | Tyr | Leu | Lys | Asn | Lys | Val | Lys | Val | Arg | |
| 10385 | | | | | 390 | | | | 395 | | | | 400 | | | |
| Pro | Gly | Asp | His | Ile | Ile | Leu | Met | Tyr | Thr | His | Ser | Glu | Glu | Phe | Val | |
| | | | | 405 | | | | 410 | | | | 415 | | | | |
| Phe | Ala | Ile | His | Ala | Cys | Ile | Ser | Leu | Gly | Ala | Ile | Val | Ile | Pro | Ile | |
| | | | | 420 | | | | 425 | | | | 430 | | | | |
| 15Ala | Pro | Leu | Asp | Gln | Asn | Arg | Leu | Asn | Glu | Asp | Val | Pro | Ala | Phe | Leu | |
| | | | | 435 | | | | 440 | | | | 445 | | | | |
| His | Ile | Val | Ser | Asp | Tyr | Asn | Val | Lys | Ala | Val | Leu | Val | Asn | Ala | Glu | |
| | | | | 450 | | | | 455 | | | | 460 | | | | |
| Val | Asp | His | Leu | Ile | Lys | Val | Lys | Pro | Val | Ala | Ser | His | Ile | Lys | Gln | |
| 20465 | | | | | 470 | | | | 475 | | | | 480 | | | |
| Ser | Ala | Gln | Val | Leu | Lys | Ile | Thr | Ser | Pro | Ala | Ile | Tyr | Asn | Thr | Thr | |
| | | | | 485 | | | | 490 | | | | 495 | | | | |
| Lys | Pro | Pro | Lys | Gln | Ser | Ser | Gly | Leu | Arg | Asp | Leu | Arg | Phe | Thr | Ile | |
| | | | | 500 | | | | 505 | | | | 510 | | | | |
| 25Asp | Pro | Ala | Trp | Ile | Arg | Pro | Gly | Tyr | Pro | Val | Ile | Val | Trp | Thr | Tyr | |
| | | | | 515 | | | | 520 | | | | 525 | | | | |
| Trp | Thr | Pro | | | | | | | | | | | | | | |
| | | | | 530 | | | | | | | | | | | | |

52

| | | | | | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|
| | 35 | | 40 | | 45 | | | | | | | | | | | | | | |
| | Gly | Tyr | Pro | Ile | Pro | Asp | Ala | Thr | Leu | Ala | Ile | Val | Asp | Pro | Glu | Thr | | | |
| | 50 | | | | | | 55 | | | | | 60 | | | | | | | |
| | Gly | Leu | Leu | Cys | Thr | Pro | Asn | Val | Val | Gly | Glu | Ile | Trp | Val | Asp | Ser | | | |
| 565 | | | | | | 70 | | | | | 75 | | | | 80 | | | | |
| | Pro | Ser | Leu | Ser | Gly | Gly | Phe | Trp | Ala | Leu | Pro | Lys | Gln | Thr | Glu | Ser | | | |
| | | | | | 85 | | | | | 90 | | | | | 95 | | | | |
| | Ile | Phe | His | Ala | Arg | Pro | Tyr | Arg | Phe | Gln | Gly | Gly | Gly | Pro | Thr | Pro | | | |
| | | | | 100 | | | | | 105 | | | | | 110 | | | | | |
| 10Val | Ile | Val | Glu | Pro | Glu | Phe | Leu | Arg | Thr | Gly | Leu | Leu | Gly | Cys | Val | | | | |
| | | | 115 | | | | | 120 | | | | | 125 | | | | | | |
| | Ile | Glu | Gly | Gln | Ile | Phe | Val | Leu | Gly | Leu | Tyr | Glu | Asp | Arg | Leu | Arg | | | |
| | | | 130 | | | | 135 | | | | | 140 | | | | | | | |
| | Gln | Lys | Val | Glu | Trp | Val | Glu | His | Gly | Val | Glu | Val | Ala | Glu | His | Arg | | | |
| 15145 | | | | | | 150 | | | | | 155 | | | 160 | | | | | |
| | Tyr | Phe | Phe | Val | Gln | His | Leu | Ile | Leu | Ser | Ile | Met | Lys | Asn | Val | Pro | | | |
| | | | | | 165 | | | | | 170 | | | | 175 | | | | | |
| | Lys | Ile | His | Asp | Cys | Ser | Ala | Phe | Asp | Val | Phe | Val | Asn | Glu | Glu | His | | | |
| | | | | 180 | | | | | 185 | | | | 190 | | | | | | |
| 20Leu | Pro | Val | Val | Val | Leu | Glu | Ser | Tyr | Thr | Ala | Ser | Thr | Ala | Pro | Val | | | | |
| | | | 195 | | | | | 200 | | | | | 205 | | | | | | |
| | Ala | Ser | Gly | Gln | Ser | Pro | Arg | Gln | Leu | Asp | Val | Pro | Leu | Leu | Asp | Ser | | | |
| | | | 210 | | | | 215 | | | | | 220 | | | | | | | |
| | Leu | Ala | Glu | Lys | Cys | Met | Gly | Val | Leu | Tyr | Gln | Glu | His | His | Leu | Arg | | | |
| 25225 | | | | | | 230 | | | | | 235 | | | 240 | | | | | |
| | Val | Tyr | Cys | Val | Met | Ile | Thr | Ala | Pro | Asn | Thr | Leu | Pro | Arg | Val | Leu | | | |
| | | | | | 245 | | | | | 250 | | | | 255 | | | | | |
| | Lys | Asn | Gly | Arg | Gln | Glu | Ile | Gly | Asn | Met | Leu | Cys | Arg | Lys | Glu | Phe | | | |
| | | | | 260 | | | | 265 | | | | | 270 | | | | | | |
| 30Asp | Asn | Gly | Ser | Leu | Pro | Cys | Glu | His | Val | Lys | Phe | Ser | Val | Glu | Arg | | | | |
| | | | | 275 | | | | 280 | | | | | 285 | | | | | | |
| | Ser | Val | Leu | Ser | Leu | Pro | Ile | Gly | Val | Asp | Pro | Val | Gly | Gly | Ile | Trp | | | |
| | | | | 290 | | | 295 | | | | | 300 | | | | | | | |
| | Ser | Val | Pro | Ser | Ser | Ala | Ala | Arg | Gln | Asp | Ala | Leu | Ala | Met | Gln | Glu | | | |
| 35305 | | | | | | 310 | | | | | 315 | | | 320 | | | | | |
| | Lys | Gln | Tyr | Ser | Gly | Val | Asp | Leu | Arg | Asp | Val | Ile | Met | Asp | Asp | Arg | | | |
| | | | | | 325 | | | | | 330 | | | | 335 | | | | | |
| | Thr | Ser | Thr | Pro | Leu | Asn | Asn | Phe | Asn | Ser | Ile | Val | Asp | Leu | Leu | Gln | | | |
| | | | | 340 | | | | 345 | | | | | 350 | | | | | | |
| 40Trp | Arg | Val | Ser | Arg | Gln | Gly | Glu | Glu | Leu | Cys | Tyr | Cys | Ser | Ile | Asp | | | | |

53

| | | |
|---|-----|-----|
| 355 | 360 | 365 |
| Gly Arg Gly Arg Glu Gly Lys Gly Ile Thr Trp Lys Lys Phe Asp Ser | | |
| 370 | 375 | 380 |
| Lys Val Ala Ala Val Ala Ala Tyr Leu Lys Asn Lys Val Lys Leu Arg | | |
| 5385 | 390 | 395 |
| Pro Gly Asp His Val Ile Leu Met Tyr Thr His Ser Glu Glu Tyr Val | | |
| | 405 | 410 |
| Phe Ala Val His Ala Cys Phe Cys Leu Gly Leu Val Ala Ile Pro Ile | | 415 |
| | 420 | 425 |
| 10Ser Pro Val Asp Gln Asn Arg Leu Ser Glu Asp Ala Pro Ala Leu Leu | | 430 |
| | 435 | 440 |
| His Val Ile Val Asp Phe Arg Val Lys Ala Ile Leu Val Asn Gly Glu | | 445 |
| | 450 | 455 |
| Val Asn Asp Leu Leu Lys Gln Lys Ile Val Ser Gln His Ile Lys Gln | | 460 |
| 15465 | 470 | 475 |
| Ser Ala His Val Val Arg Thr Ser Val Pro Ser Val Tyr Asn Thr Ser | | 480 |
| | 485 | 490 |
| Lys Pro Pro Lys Gln Ser His Gly Cys Arg His Leu Gly Phe Thr Met | | 495 |
| | 500 | 505 |
| 20Asn Pro Gln Trp Leu Asn Ser Lys Gln Pro Ala Val Ile Trp Thr Tyr | | 510 |
| | 515 | 520 |
| Trp Thr Pro | | 525 |
| | 530 | |

25<210> 55

<211> 2073

<212> DNA

<213> Cochliobolus heterostrophus

30<400> 55

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| gccccgtggg gtcgggatgg gtagcgctgc aggggttttag ctgagatgga ggggagagag | 120 |
| gggggggttg ggatgtttaa aaggatgggg aggggtgtgt tctgtgctt ggatgttacg | 180 |
| ctgttgcgct gcttacttgc tacgttgctc gtggcagccg actcagtctt tctacctgct | 240 |
| 35ttcttttgct ctgtctcttt tttttattta cttggggcct ttgagatagc tcagagagggc | 300 |
| gaaaggggtt gagataagag acgggtgcga atagagggcg agtacgatga gcgtggataa | 360 |
| aatgcaggat gaaaagggtt agcggagtgg gagtgagggg tttgaagagg ggcttctgga | 420 |
| ggatccgaag gcaacgagta gggtgtgtt caagatcgat tgtcggtatg tttctctctc | 480 |
| cattctctgct ctccatgtct ttatcttgag ggcttttgtg gatgatgtac catcctgccg | 540 |
| 40gttctcgccc tgctgttcct gtgctcgttc attgatcgta caaaccttgg gaatgcgaag | 600 |

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attcttgggtt tggagaatga tctccatctt acggaccacc agtacgctat tgggctttgc      660
gtctttttacg ctacgtatat tgcgaggtaa gcttcctgta tggcagatgc agtccagaag      720
actaaatttg tgcagcgaac tcccggtccaa tttgctgctg aaaaagggtat cgccaaagat      780
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5aaatttgcgc tcttttgctt ctgttcgcgc gctcctgggc gttgctgaag gaggcctatt      900
gcctggaatg gtaagatttt ggcgacgtaa taaaccgtct ttcgctaacg ccttgctagg      960
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tactgcagcc tctctatctg gtgcttttgg cggactcctc gctcgaggcc tcaatgccat     1080
tggcccagca agcggactcg aaggctggag atggatcctg atagttgagg gcttgataac     1140
10cgttggcgtc ggcgcatgct ctgctatctt ccttcccaat tccatcgaat cagccggttt     1200
ccttagcccc tccgaaaaag cccacgcccg cttccgactc ggtgaagcat ccgcctcgca     1260
cgaacgcttc gactgggccc aaatcaaacg cggcatcttc aacctccaag tctggctcac     1320
agccactgcc tacttctcta tcctctcagg cctctactcc ttcggcctct tcctccccac     1380
aatcatcaac aacggcttcg ccaaggaccc caacaaagcc cagctctgga ccgtcattcc     1440
15ttacgcgctc gcttccgtct tcaccgtcct tgtagccatt ctctccgacc gcctcgctct     1500
acgtggccca gtcattgctgt gtacccttcc cgttgctatc atcggctacg gagtcatcag     1560
ccaatcgacg aaccgaaaag tacaatacgg aatgacattt ctcatggcta caggcatgta     1620
ttcctccgtc ccatgtattc tttcttggaa cagcaataat tccgctggcc actacaagcg     1680
cgcgactaca tcggcgctgc agcttgcatg tgccaatgcg ggttggttcg tcgagcgtt     1740
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aggtggattt ccatcgtttt gctggcggga tatgcagcta acgtgaatga tagggtcgca     1920
gcgaatgtgg cgtgggtgtg gaaaatcaac cgcgataagg cgagtggaaa gtatgcggaa     1980
ttcgaaggac gaggagatga tagggatccg gcgtttaaga tggatgatga agggattttg     2040
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<210> 56

<211> 487

<212> PRT

30<213> Cochliobolus heterostrophus

<400> 56

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35Gly Leu Leu Glu Asp Pro Lys Ala Thr Ser Arg Leu Leu Phe Lys Ile
      20              25              30
Asp Cys Arg Tyr Val Ser Leu Ser Ile Pro Ala Leu His Val Phe Ile
      35              40              45
Leu Arg Ala Phe Val Asp Asp Val Pro Ser Cys Arg Phe Ser Pro Cys
40      50              55              60

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55

Cys Ser Cys Ala Arg Ser Leu Ile Val Gln Thr Leu Gly Met Arg Arg
 65 70 75 80
 Phe Leu Val Trp Arg Met Ile Ser Ile Leu Arg Thr Thr Ser Thr Leu
 85 90 95
 5Leu Gly Phe Ala Ser Phe Thr Leu Arg Ile Leu Arg Gly Lys Leu Pro
 100 105 110
 Val Trp Gln Met Gln Ser Arg Arg Leu Asn Leu Cys Ser Glu Leu Pro
 115 120 125
 Ser Asn Leu Leu Leu Lys Lys Val Ser Pro Lys Ile Trp Leu Pro Phe
 10 130 135 140
 Leu Thr Ala Ile Trp Gly Val Leu Thr Met Cys Leu Gly Phe Val Thr
 145 150 155 160
 Asn Phe Ala Ser Phe Ala Ser Val Arg Ala Leu Leu Gly Val Ala Glu
 165 170 175
 15Gly Gly Leu Leu Pro Gly Met Val Arg Phe Trp Arg Arg Asn Lys Pro
 180 185 190
 Ser Phe Ala Asn Ala Leu Leu Gly Leu Tyr Leu Ser His Phe Tyr Arg
 195 200 205
 Arg Gln Glu Leu Ala Leu Arg Ile Gly Ile Phe Tyr Thr Ala Ala Ser
 20 210 215 220
 Leu Ser Gly Ala Phe Gly Gly Leu Leu Ala Arg Gly Leu Asn Ala Ile
 225 230 235 240
 Gly Pro Ala Ser Gly Leu Glu Gly Trp Arg Trp Ile Leu Ile Val Glu
 245 250 255
 25Gly Leu Ile Thr Val Gly Val Gly Ala Cys Ser Ala Ile Phe Leu Pro
 260 265 270
 Asn Ser Ile Glu Ser Ala Gly Phe Leu Ser Pro Ser Glu Lys Ala His
 275 280 285
 Ala Arg Phe Arg Leu Gly Glu Ala Ser Ala Ser His Glu Arg Phe Asp
 30 290 295 300
 Trp Ala Glu Ile Lys Arg Gly Ile Phe Asn Leu Gln Val Trp Leu Thr
 305 310 315 320
 Ala Thr Ala Tyr Phe Ser Ile Leu Ser Gly Leu Tyr Ser Phe Gly Leu
 325 330 335
 35Phe Leu Pro Thr Ile Ile Asn Asn Gly Phe Ala Lys Asp Pro Asn Lys
 340 345 350
 Ala Gln Leu Trp Thr Val Ile Pro Tyr Ala Val Ala Ser Val Phe Thr
 355 360 365
 Val Leu Val Ala Ile Leu Ser Asp Arg Leu Ala Leu Arg Gly Pro Val
 40 370 375 380

56

Met Leu Cys Thr Leu Pro Val Ala Ile Ile Gly Tyr Gly Val Ile Ser
 385 390 395 400
 Gln Ser Thr Asn Pro Lys Val Gln Tyr Gly Met Thr Phe Leu Met Ala
 405 410 415
 5Thr Gly Met Tyr Ser Ser Val Pro Cys Ile Leu Ser Trp Asn Ser Asn
 420 425 430
 Asn Ser Ala Gly His Tyr Lys Arg Ala Thr Thr Ser Ala Leu Gln Leu
 435 440 445
 Ala Ile Ala Asn Ala Gly Trp Phe Val Ala Ser Phe Thr Tyr Gln Lys
 10 450 455 460
 Ser Glu Lys Pro Asn Phe His Lys Ser His Ser Ile Met Leu Gly Leu
 465 470 475 480
 Leu Cys Ala Ala Trp Val Leu
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15

<210> 57

<211> 1900

<212> DNA

<213> *Cochliobolus heterostrophus*

20

<400> 57

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 tagggctgca tgcagtgcgc atcatctgca tgcacttgct gtgccaagtc gtgtactaca 180
 25caagtgcgag ttgctatctg taacgaggaa ccttgtatct aaaagtgtat acgtgaggta 240
 cgtgtgttcc agacctcaa atctaaagct actaaaacaa tagaaacagc ggagtctact 300
 ccgacaaggt caagtgaaag gcggcggcat aaaagtcaat cgaatcaaag tacacggaca 360
 tacgagcaat ctacacacgg tcatggctat agcttacttt cgttctgctt caatcgatg 420
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 gaccatcacc agcccaaggg gtctccatct cggatgaagat ccattcatct gcgcggaaac 600
 tgcgaggatt gtgaaagtag atggtgtggt ccagactaac catcatgcca atctcaggct 660
 ttgcgtctcc gctctttgcc aggtcttctg ctttcctcaa ttcacgatg cgtgcttgt 720
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 cggagagatg ggcttcgtga ccgccagcgg gggagatctt accgcgagcc tttatccatt 960
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57

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aacgactttc ccgagcgcac cttttctaca tggatatatga cggggatctc ggagttgcct 1320
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15<210> 58

<211> 368

<212> PRT

<213> Cochliobolus heterostrophus

20<400> 58

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      20             25             30
25Ile Phe Thr Asn Thr Arg Pro Leu Trp His Pro Pro Gly Ala Arg Gly
      35             40             45
Ile Phe Gly Gly Ala Ala Ile Ala Gln Thr Leu Ser Ala Ala Gln Lys
      50             55             60
Thr Val Asp Pro Asp Phe Thr Val His Ser Met His Cys Tyr Phe Ile
3065             70             75             80
Leu Ala Gly Asn Ser Glu Ile Pro Val Ile Tyr His Val Glu Arg Val
      85             90             95
Arg Ser Gly Lys Ser Phe Ala Thr Arg Thr Val Gln Ala Arg Gln Arg
      100            105            110
35Gly Asn Val Ile Phe Thr Thr Thr Met Ser Phe Val Arg Gln Asn Ser
      115            120            125
Gly Gly Ala Gln Lys Val Glu His Ile Tyr Pro Met Pro Asp Val Pro
      130            135            140
Ala Pro Lys Glu Gly Ser Asp Asp Leu Lys Thr Pro Asn Asp Gly Gln
40145            150            155            160

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58

Ser Pro Phe Gln Thr Gln Leu Leu Pro Ile Glu Asn Ala Asp Asp Ser
 165 170 175
 Asp Lys Pro His Thr Lys Lys Cys Arg Gln Trp Ile Lys Ala Arg Gly
 180 185 190
 5Lys Ile Ser Pro Ala Gly Gly His Glu Ala His Leu Ser Ala Ile Ala
 195 200 205
 Tyr Met Ser Asp Ser Tyr Phe Ile Gly Thr Val Ala Arg Ala His Lys
 210 215 220
 Leu Leu Arg Tyr Ser Asn Gln Arg Lys Ser Arg Ala Arg Ser Ser Ile
 10225 230 235 240
 Asp Glu Asp Val Leu Lys Lys Leu Leu Glu Met Asp Asp Ala Glu Leu
 245 250 255
 Gln Arg Gln Ser Phe Val Asn Glu Ser Asp Lys Gln Arg Ile Arg Glu
 260 265 270
 15Leu Arg Lys Ala Glu Asp Leu Ala Lys Ser Gly Asp Ala Lys Pro Glu
 275 280 285
 Ile Gly Met Met Val Ser Leu Asp His Thr Ile Tyr Phe His Asn Pro
 290 295 300
 Arg Ser Phe Arg Ala Asp Glu Trp Ile Phe Thr Glu Met Glu Thr Pro
 20305 310 315 320
 Trp Ala Gly Asp Gly Arg Gly Leu Val Ser Gln Arg Met Tyr Thr Lys
 325 330 335
 Asp Gly Thr Leu Ile Ala Ser Cys Val Gln Glu Val Ser Ser Leu Leu
 340 345 350
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<210> 59

<211> 42115

30<212> DNA

<213> Cochliobolus heterostrophus

<220>

<221> misc_feature

35<222> (1)...(42115)

<223> n = any any nucleotide

<400> 59

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| | | | | | | | |
|----|-------------|-------------|-------------|-------------|-------------|-------------|------|
| | aagcgagctt | ttcgtaatat | cgaaagcatc | ttgcgctgct | caaaatctga | gaaaatggtc | 180 |
| | ctactaggca | gaagcaagac | agtataatg | ggcttcccag | agccaccttg | gagctaagcc | 240 |
| | gtttgoggac | gcctgcattc | aacgccaact | cgctacgctt | ctttggagac | aacgtctttc | 300 |
| | cttgtcagat | gatcgacact | gcgttgatga | ctcgaaccag | ttgggagggtg | tagttcctcc | 360 |
| | 5tttattttat | tgagacatca | tggcgacagc | tgtattgctt | gcccgtatgc | tctttcctac | 420 |
| | taacagcacg | attgcttact | atgtgaagac | tcgttggaca | tgagcgctt | accaacaaat | 480 |
| | actcccactc | tatagaaaga | agtcgaggta | aagtgaagtc | aagtgaagtg | aacaagcatt | 540 |
| | cactgtatgc | tttaggcagc | tccgaccaag | tgtatagaag | gctgcatcat | ctgccattcc | 600 |
| | acctctccac | cttctgcttt | tcgccgatcc | ctcttgcttt | acttagaacg | ctcctgatat | 660 |
| 10 | ccgttccctc | tacaaaatcg | agaaggtct | gtatcaggaa | cacagccatg | gaggactcgt | 720 |
| | gtctttgcac | ttcactctca | cagccaccgg | agcttgaact | gacaagattg | ccatctttcg | 780 |
| | ataccacac | caatatgtcg | aagcaacaga | aaaacagaga | tatgacttgg | cagcaacagg | 840 |
| | cagacttcgt | gcaggccaca | gtaagtgaag | taagccttat | gaccgcatca | tgtctcacta | 900 |
| | tcctttcgta | atcatacacc | aaccgtggtg | aaagccacag | tagacttttc | tagcaccttc | 960 |
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1 5 10 15
Arg Xaa Ile

30<210> 75

<211> 19

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<213> Cochliobolus heterostrophus

35<400> 75

Phe Ser Arg Ala Pro Thr Gly Asp Leu Arg Gly Val Val Leu Ser His
1 5 10 15
Arg Thr Ile

40

81

<210> 76

<211> 18

<212> PRT

<213> Cochliobolus heterostrophus

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<400> 76

Trp Thr Tyr Trp Thr Pro Asp Gln Arg Ala Val Gln Leu Gly His Ser

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15

Gln Ile

10

<210> 77

<211> 19

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15<213> Myxococcus xanthus

<400> 77

Tyr Thr Ser Gly Ser Thr Ala Asp Pro Lys Gly Val Val Leu Thr His

1

5

10

15

20Arg Asn Leu

<210> 78

<211> 19

25<212> PRT

<213> Bacillus brevis

<400> 78

Tyr Thr Ser Gly Thr Thr Gly Asn Pro Lys Gly Thr Met Leu Glu His

30 1

5

10

15

Lys Gly Ile

<210> 79

35<211> 19

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<213> Cochliobolus carbonum

<400> 79

40Phe Thr Ser Gly Ser Thr Gly Val Pro Lys Cys Ile Val Val Thr His

82

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| Ser | Gln | Ile | |

5<210> 80

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<213> Cochliobolus carbonum

10<400> 80

Phe Thr Ser Gly Thr Gly Val Pro Lys Gly Ala Val Ala Thr His Gln

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| 1 | 5 | 10 | 15 |
|---|---|----|----|

Ala Tyr

15

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<213> Fusarium scirpi

20

<400> 81

Phe Thr Ser Gly Ser Thr Gly Ile Pro Lys Gly Ile Met Ile Glu His

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|---|---|----|----|
| 1 | 5 | 10 | 15 |
|---|---|----|----|

Arg Ser Phe

25

<210> 82

<211> 19

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30<213> Fusarium scirpi

<400> 82

Phe Thr Ser Gly Ser Thr Gly Lys Pro Lys Gly Val Met Ile Glu His

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| 1 | 5 | 10 | 15 |
|---|---|----|----|

35Arg Ala Ile

<210> 83

<211> 19

40<212> PRT

83

<213> Aspergillus nidulans

<400> 83

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5 1 5 10 15
Thr Asn Val

<210> 84

10<211> 19

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<213> Aspergillus nidulans

<400> 84

15Tyr Thr Ser Gly Thr Thr Gly Arg Pro Lys Gly Val Thr Val Glu His
1 5 10 15
His Gly Val

20<210> 85

<211> 19

<212> PRT

<213> Tolypocladium nivenm

25<400> 85

Phe Thr Ser Gly Ser Thr Gly Lys Pro Lys Gly Val Met Ile Glu His
1 5 10 15
Arg Gly Ile

30

<210> 86

<211> 19

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<213> Tolypocladium nivenm

35

<400> 86

Phe Thr Ser Gly Ser Thr Gly Lys Pro Lys Gly Val Met Ile Glu His
1 5 10 15
Arg Gly Val

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84

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<211> 14
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15 1 5 10

<210> 88
<211> 14
<212> PRT
20<213> Cochliobolus heterostrophus

<400> 88
Gly Glu Ile Trp Val Asp Ser Pro Ser Leu Ser Gly Gly Phe
1 5 10
25
<210> 89
<211> 14
<212> PRT
<213> Cochliobolus heterostrophus
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<400> 89
Gly Glu Ile Trp Val Gln Ser Glu Ala Asn Ala Tyr Ser Phe
1 5 10

35<210> 90
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<213> Myxococcus xanthus

40<400> 90

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Gly Glu Ile Trp Val Arg Gly Pro Ser Val Ala Gln Gly Tyr

1 5 10

<210> 91

5<211> 14

<212> PRT

<213> Bacillus brevis

<400> 91

10Gly Glu Leu Cys Ile Gly Gly Glu Gly Leu Ala Arg Gly Tyr

1 5 10

<210> 92

<211> 14

15<212> PRT

<213> Cochliobolus carbonum

<400> 92

Gly Glu Leu Leu Ile Glu Ser Gly His Leu Ala Asp Lys Tyr

20 1 5 10

<210> 93

<211> 14

<212> PRT

25<213> Cochliobolus carbonum

<400> 93

Gly Glu Leu Ile Ile Glu Gly Ser Ile Leu Cys Arg Gly Tyr

1 5 10

30

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<212> PRT

<213> Fusarium scirpi

35

<400> 94

Gly Glu Leu Val Ile Glu Ser Ala Gly Ile Ala Arg Asp Tyr

1 5 10

40<210> 95

86

<211> 14

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<213> Fusarium scirpi

5<400> 95

Gly Glu Leu Val Val Thr Gly Asp Gly Val Gly Arg Gly Tyr

1

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10

<210> 96

10<211> 14

<212> PRT

<213> Aspergillus nidulans

<400> 96

15Gly Glu Leu His Ile Gly Gly Leu Gly Ile Ser Lys Gly Tyr

1

5

10

<210> 97

<211> 14

20<212> PRT

<213> Aspergillus nidulans

<400> 97

Gly Glu Leu Tyr Leu Gly Gly Glu Gly Val Val Arg Gly Tyr

25 1

5

10

<210> 98

<211> 14

<212> PRT

30<213> Tolypocladium nivenm

<400> 98

Gly Glu Leu Val Val Ser Gly Asp Gly Leu Ala Arg Gly Tyr

1

5

10

35

<210> 99

<211> 14

<212> PRT

<213> Tolypocladium nivenm

40

87

<400> 99

Gly Glu Leu Val Val Thr Gly Asp Gly Leu Ala Arg Gly Tyr

1

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10

5<210> 100

<211> 8

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15<223> Xaa = any amino acid

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<211> 9

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Phe Leu Arg Thr Gly Leu Leu Gly Phe

1

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30<210> 102

<211> 9

<212> PRT

<213> Cochliobolus heterostrophus

35<400> 102

Tyr Val Arg Thr Gly Asp Leu Gly Phe

1

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<210> 103

40<211> 9

<212> PRT

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<400> 103

5Trp Leu Arg Thr Gly Asp Leu Gly Phe

1

5

<210> 104

<211> 8

10<212> PRT

<213> *Bacillus brevis*

<400> 104

Tyr Lys Thr Gly Asp Gln Ala Arg

15 1

5

<210> 105

<211> 8

<212> PRT

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<400> 105

Tyr Arg Thr Gly Asp Leu Val Arg

1

5

25

<210> 106

<211> 8

<212> PRT

<213> *Cochliobolus carbonum*

30

<400> 106

Tyr Lys Thr Gly Asp Leu Val Arg

1

5

35<210> 107

<211> 8

<212> PRT

<213> *Fusarium scirpi*

40<400> 107

89

Tyr Arg Thr Gly Asp Leu Ala Cys
1 5

<210> 108

5<211> 8

<212> PRT

<213> *Fusarium scirpi*

<400> 108

10Tyr Arg Thr Gly Asp Arg Met Arg
1 5

<210> 109

<211> 8

15<212> PRT

<213> *Aspergillus nidulans*

<400> 109

Tyr Lys Thr Gly Asp Leu Ala Arg
20 1 5

<210> 110

<211> 8

<212> PRT

25<213> *Aspergillus nidulans*

<400> 110

Tyr Lys Thr Gly Asp Leu Val Arg
1 5
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<210> 111

<211> 8

<212> PRT

<213> *Tolypocladium nivenm*

35

<400> 111

Tyr Arg Thr Gly Asp Arg Ala Arg
1 5

40<210> 112

90

<211> 8

<212> PRT

<213> Tolypocladium nivenm

5<400> 112

Tyr Arg Thr Gly Asp Arg Ala Arg

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5

<210> 113

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<222> 4, 6, 13

<223> Xaa = any amino acid

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1

5

10

15

Leu Gly Glu Val Glu

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<210> 114

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30<213> Cochliobolus heterostrophus

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Leu Gly Leu Tyr Glu Asp Arg Ile Arg Gln Arg Val Glu Asn Gly Gln

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35Leu Glu

<210> 115

<211> 21

40<212> PRT

91

<213> *Bacillus brevis*

<400> 115

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 5 1 5 10 15
 Leu Glu Glu Val Glu
 20

<210> 116

10<211> 21

<212> PRT

<213> *Cochliobolus carbonum*

<400> 116

15Leu Gly Arg Lys Asp Thr Gln Val Lys Met Asn Gly Gln Arg Phe Glu
 1 5 10 15
 Leu Gly Glu Val Glu
 20

20<210> 117

<211> 21

<212> PRT

<213> *Cochliobolus carbonum*

25<400> 117

Val Gly Arg Ser Asp Thr Gln Ile Lys Leu Ala Gly Gln Arg Val Glu
 1 5 10 15
 Leu Gly Asp Val Glu
 20

30

<210> 118

<211> 21

<212> PRT

<213> *Fusarium scirpi*

35

<400> 118

Leu Gly Arg Met Asp Ser Gln Val Lys Ile Arg Gly Gln Arg Val Glu
 1 5 10 15
 Leu Gly Ala Val Glu
 40 20

92

<210> 119

<211> 21

<212> PRT

<213> *Fusarium scirpi*

5

<400> 119

Phe Gly Arg Met Asp Asn Gln Phe Lys Ile Arg Gly Asn Arg Ile Glu

1

5

10

15

Ala Gly Glu Val Glu

10

20

<210> 120

<211> 21

<212> PRT

15<213> *Aspergillus nidulans*

<400> 120

Leu Gly Arg Ala Asp Phe Gln Ile Lys Leu Arg Gly Ile Arg Ile Glu

1

5

10

15

20Pro Gly Glu Ile Glu

20

<210> 121

<211> 21

25<212> PRT

<213> *Aspergillus nidulans*

<400> 121

Leu Gly Arg Asn Asp Phe Gln Val Lys Ile Arg Gly Leu Arg Ile Glu

30 1

5

10

15

Leu Gly Glu Ile Glu

20

<210> 122

35<211> 21

<212> PRT

<213> *Tolypocladium nivenm*

<400> 122

40Phe Gly Arg Met Asp Gln Gln Val Lys Ile Arg Gly His Arg Ile Glu

93

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|-----|-----|-----|---------|
| 1 | 5 | 10 | 15 |
| Pro | Ala | Glu | Val Glu |
| 20 | | | |

5<210> 123

<211> 21

<212> PRT

<213> Tolypocladium nivenm

10<400> 123

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| Phe | Gly | Arg | Met | Asp | His | Gln | Val | Lys | Val | Arg | Gly | His | Arg | Ile | Glu |
| 1 | | | | | 5 | | | | | 10 | | | | | 15 |
| Leu | Ala | Glu | Val | Glu | | | | | | | | | | | |
| 20 | | | | | | | | | | | | | | | |

15

<210> 124

<211> 21

<212> PRT

<213> Cochliobolus heterostrophus

20

<400> 124

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| Leu | Gly | Ser | Ile | Gly | Asp | Thr | Phe | Glu | Val | Asn | Gly | Leu | Asn | His | Phe |
| 1 | | | | | | | | | | | | | | | |
| Ser | Met | Asp | Ile | Glu | | | | | | | | | | | |
| 25 | 20 | | | | | | | | | | | | | | |

<210> 125

<211> 21

<212> PRT

30<213> Myxococcus xanthus

<400> 125

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Gly | Arg | Arg | Lys | Asp | Leu | Leu | Val | Ile | Arg | Gly | Arg | Asn | Tyr | Tyr |
| 1 | | | | | | | | | | | | | | | |
| 35 | Pro | Gln | Asp | Leu | Glu | | | | | | | | | | |
| 20 | | | | | | | | | | | | | | | |

<210> 126

<211> 13

40<212> PRT

94

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10<400> 126

Phe Phe Xaa Xaa Gly Gly Asp Ser Leu Xaa Ala Xaa Xaa

1

5

10

<210> 127

15<211> 13

<212> PRT

<213> Cochliobolus heterostrophus

<400> 127

20Leu Asp Ile Pro Phe Leu Asp Ser Leu Ser Glu Arg Cys

1

5

10

<210> 128

<211> 13

25<212> PRT

<213> Cochliobolus heterostrophus

<400> 128

Arg Asp Pro Asn Gly Gln Asp Ser Gln Met Ile Thr Glu

30 1

5

10

<210> 129

<211> 13

<212> PRT

35<213> Myxococcus xanthus

<400> 129

Leu Pro Asp Leu Gly Leu Asp Ser Leu Ala Leu Val Glu

1

5

10

40

95

<210> 130

<211> 13

<212> PRT

<213> *Bacillus brevis*

5

<400> 130

Phe Tyr Ala Leu Gly Gly Asp Ser Ile Lys Ala Ile Gln

1

5

10

10<210> 131

<211> 13

<212> PRT

<213> *Cochliobolus carbonum*

15<400> 131

Phe Ile His Ala Gly Gly Asp Ser Ile Thr Ala Met Gln

1

5

10

<210> 132

20<211> 13

<212> PRT

<213> *Cochliobolus carbonum*

<400> 132

25Phe Phe Ser Ser Gly Gly Asn Ser Met Ala Ala Ile Ala

1

5

10

<210> 133

<211> 13

30<212> PRT

<213> *Fusarium scirpi*

<400> 133

Phe Phe Glu Met Gly Gly Asn Ser Ile Ile Ala Ile Lys

35 1

5

10

<210> 134

<211> 13

<212> PRT

40<213> *Fusarium scirpi*

96

<400> 134

Phe Phe Gln Leu Gly Gly His Ser Leu Leu Ala Thr Lys

1 5 10

5<210> 135

<211> 13

<212> PRT

<213> *Aspergillus nidulans*

10<400> 135

Phe Phe Arg Leu Gly Gly His Ser Ile Thr Cys Ile Gln

1 5 10

<210> 136

15<211> 13

<212> PRT

<213> *Aspergillus nidulans*

<400> 136

20Phe Phe Ser Leu Gly Gly Asp Ser Leu Lys Ser Thr Lys

1 5 10

<210> 137

<211> 13

25<212> PRT

<213> *Tolypocladium nivenm*

<400> 137

Phe Phe Asp Leu Gly Gly His Ser Leu Thr Ala Met Lys

30 1 5 10

<210> 138

<211> 13

<212> PRT

35<213> *Tolypocladium nivenm*

<400> 138

Phe Phe Asn Val Gly Gly His Ser Leu Leu Ala Thr Lys

1 5 10

40

97

<210> 139
<211> 16
<212> PRT
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<223> Xaa = any amino acid

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<210> 140
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<212> PRT
20<213> Cochliobolus heterostrophus

<400> 140
Val Leu Arg Pro Gly Pro Ser Ser Gly Ser Glu Gln His Asp Gln Ala
1 5 10 15
25
<210> 141
<211> 16
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<213> Aspergillus nidulans
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Tyr His Phe Ile Gly Trp Ser Phe Gly Gly Thr Ile Ala Met Glu Ile
1 5 10 15

35<210> 142
<211> 16
<212> PRT
<213> Bacillus brevis

40<400> 142

98

Tyr Val Leu Ile Gly Tyr Ser Ser Gly Gly Asn Leu Ala Phe Glu Val
1 5 10 15

<210> 143

5<211> 16

<212> PRT

<213> Bacillus brevis

<400> 143

10Phe Ala Phe Leu Gly His Ser Met Gly Ala Leu Ile Ser Phe Glu Leu
1 5 10 15

<210> 144

<211> 16

15<212> PRT

<213> Myxococcus xanthus

<400> 144

Leu Thr Leu Phe Gly Tyr Ser Ala Gly Cys Ser Leu Ala Phe Glu Ala
20 1 5 10 15

<210> 145

<211> 16

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25<213> Brevibacillus brevis

<400> 145

Tyr Thr Leu Met Gly Tyr Ser Ser Gly Gly Asn Leu Ala Phe Glu Val
1 5 10 15

30

<210> 146

<211> 16

<212> PRT

<213> Brevibacillus brevis

35

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Phe Ala Phe Phe Gly His Ser Met Gly Gly Leu Val Ala Phe Glu Leu
1 5 10 15

40<210> 147

99

<211> 5
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<210> 149
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30<213> Artificial Sequence

<220>
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35<400> 149
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<210> 150
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19

18

100

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<223> Primer

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17

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<400> 151

gtctctatct agctacggca ttgt

24

20<210> 152

<211> 20

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25<220>

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gacgggccgc tagtatccat

20

30

<210> 153

<211> 23

<212> DNA

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35

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40acgtctcaag tcaatgccca ata

23

101

<210> 154

<211> 24

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18

<210> 156

<211> 18

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40<223> Primer

102

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<400> 158
ttccagcgcg taagtaagtc a 21

15<210> 159
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20<220>
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103

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<223> Primer

<400> 161

5catggcgact ccctggt

17

<210> 162

<211> 22

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25

<400> 163

acatatagtt tggacgctct gc

22

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30<211> 24

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35<223> Primer

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24

40<210> 165

104

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<223> Primer

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19

10

<210> 166

<211> 20

<212> DNA

<213> Artificial Sequence

15

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<211> 20

35<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

40

105

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<220>
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<400> 169
atcaggcgag aagggtgtg 19

15<210> 170
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<212> DNA
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25
<210> 171
<211> 19
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30
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35tgggcagcaa atggcacag 19

<210> 172
<211> 18
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40<213> Artificial Sequence

106

<220>

<223> Primer

<400> 172

5gcgctgcaca acccatca

18

<210> 173

<211> 22

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<223> Primer

15<400> 173

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22

<210> 174

<211> 21

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<213> Artificial Sequence

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25

<400> 174

tgtgttgacc tccactagct c

21

<210> 175

30<211> 21

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35<223> Primer

<400> 175

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21

40<210> 176

107

<211> 21

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5<220>

<223> Primer

<400> 176

ctctcaaccc acaacctaac c

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<210> 177

<211> 22

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<211> 21

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21

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<223> Primer

40

108

<400> 179
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<400> 180
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15<210> 181
<211> 21
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20<220>
<223> Primer

<400> 181
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<210> 182
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<400> 182
35tttgggtccg aagtagagat t 21

<210> 183
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40<213> Artificial Sequence

109

<220>

<223> Primer

<400> 183

5ggcaagaatc gaccctacc

19

<210> 184

<211> 711

<212> PRT

10<213> *Saccharomyces cerevisiae*

<400> 184

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Leu Val Leu Thr Asp Ala Cys Met Gly Val Leu Gly Glu Val Thr Trp
      35             40             45
Glu Tyr Ser Ser Asp Asp Leu Tyr Ser Ser Pro Ala Cys Thr Tyr Glu
20  50             55             60
Pro Ala Leu Gln Ser Met Leu Tyr Cys Ile Tyr Glu Ser Leu Asn Glu
65             70             75             80
Lys Gly Tyr Ser Asn Arg Thr Phe Glu Lys Thr Phe Ala Ala Ile Lys
      85             90             95
25Glu Asp Cys Ala Tyr Tyr Thr Asp Asn Leu Gln Asn Met Thr Asn Ala
      100             105             110
Asp Phe Tyr Asn Met Leu Asn Asn Gly Thr Thr Tyr Ile Ile Gln Tyr
      115             120             125
Ser Glu Gly Ser Ala Asn Leu Thr Tyr Pro Ile Glu Met Asp Ala Gln
30  130             135             140
Val Arg Glu Asn Tyr Tyr Tyr Ser Tyr His Gly Phe Tyr Ala Asn Tyr
145             150             155             160
Asp Ile Gly His Thr Tyr Gly Gly Ile Ile Cys Ala Tyr Phe Val Gly
      165             170             175
35Val Met Ile Leu Ala Ser Ile Leu His Tyr Leu Ser Tyr Thr Pro Phe
      180             185             190
Lys Thr Ala Leu Phe Lys Gln Arg Leu Val Arg Tyr Val Arg Arg Tyr
      195             200             205
Leu Thr Ile Pro Thr Ile Trp Gly Lys His Ala Ser Ser Phe Ser Tyr
40  210             215             220

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110

Leu Lys Ile Phe Thr Gly Phe Leu Pro Thr Arg Ser Glu Gly Val Ile
 225 230 235 240
 Ile Leu Gly Tyr Leu Val Leu His Thr Val Phe Leu Ala Tyr Gly Tyr
 245 250 255
 5Gln Tyr Asp Pro Tyr Asn Leu Ile Phe Asp Ser Arg Arg Glu Gln Ile
 260 265 270
 Ala Arg Tyr Val Ala Asp Arg Ser Gly Val Leu Ala Phe Ala His Phe
 275 280 285
 Pro Leu Ile Ala Leu Phe Ala Gly Arg Asn Asn Phe Leu Glu Phe Ile
 10 290 295 300
 Ser Gly Val Lys Tyr Thr Ser Phe Ile Met Phe His Lys Trp Leu Gly
 305 310 315 320
 Arg Met Met Phe Leu Asp Ala Val Ile His Gly Ala Ala Tyr Thr Ser
 325 330 335
 15Tyr Ser Val Phe Tyr Lys Asp Trp Ala Ala Ser Lys Glu Glu Thr Tyr
 340 345 350
 Trp Gln Phe Gly Val Ala Ala Leu Cys Ile Val Gly Val Met Val Phe
 355 360 365
 Phe Ser Leu Ala Met Phe Arg Lys Phe Phe Tyr Glu Ala Phe Leu Phe
 20 370 375 380
 Leu His Ile Val Leu Gly Ala Leu Phe Phe Tyr Thr Cys Trp Glu His
 385 390 395 400
 Val Val Glu Leu Ser Gly Ile Glu Trp Ile Tyr Ala Ala Ile Ala Ile
 405 410 415
 25Trp Thr Ile Asp Arg Leu Ile Arg Ile Val Arg Val Ser Tyr Phe Gly
 420 425 430
 Phe Pro Lys Ala Ser Leu Gln Leu Val Gly Asp Asp Ile Ile Arg Val
 435 440 445
 Thr Val Lys Arg Pro Val Arg Leu Trp Lys Ala Lys Pro Gly Gln Tyr
 30 450 455 460
 Val Phe Val Ser Phe Leu His His Leu Tyr Phe Trp Gln Ser His Pro
 465 470 475 480
 Phe Thr Val Leu Asp Ser Ile Ile Lys Asp Gly Glu Leu Thr Ile Ile
 485 490 495
 35Leu Lys Glu Lys Lys Gly Val Thr Lys Leu Val Lys Lys Tyr Val Cys
 500 505 510
 Cys Asn Gly Gly Lys Ala Ser Met Arg Leu Ala Ile Glu Gly Pro Tyr
 515 520 525
 Gly Ser Ser Ser Pro Val Asn Asn Tyr Asp Asn Val Leu Leu Leu Thr
 40 530 535 540

111

Gly Gly Thr Gly Leu Pro Gly Pro Ile Ala His Ala Ile Lys Leu Gly
 545 550 555 560
 Lys Thr Ser Ala Ala Thr Gly Lys Gln Phe Ile Lys Leu Val Ile Ala
 565 570 575
 5Val Arg Gly Phe Asn Val Leu Glu Ala Tyr Lys Pro Glu Leu Met Cys
 580 585 590
 Leu Glu Asp Leu Asn Val Gln Leu His Ile Tyr Asn Thr Met Glu Val
 595 600 605
 Pro Ala Leu Thr Pro Asn Asp Ser Leu Glu Ile Ser Gln Gln Asp Glu
 10 610 615 620
 Lys Ala Asp Gly Lys Gly Val Val Met Ala Thr Thr Leu Glu Gln Ser
 625 630 635 640
 Pro Asn Pro Val Glu Phe Asp Gly Thr Val Phe His His Gly Arg Pro
 645 650 655
 15Asn Val Glu Lys Leu Leu His Glu Val Gly Asp Leu Asn Gly Ser Leu
 660 665 670
 Ala Val Val Cys Cys Gly Pro Pro Val Phe Val Asp Glu Val Arg Asp
 675 680 685
 Gln Thr Ala Asn Leu Val Leu Glu Lys Pro Ala Lys Ala Ile Glu Tyr
 20 690 695 700
 Phe Glu Glu Tyr Gln Ser Trp
 705 710

<210> 185

25<211> 1774

<212> PRT

<213> Cochliobolus heterostrophus

<400> 185

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 Glu Val Asn Gln Gly Tyr Phe Ser Asp Phe Thr Gly Gln Gln Met Gln
 35 35 40 45
 Asp Asn Arg Asp Ser Tyr Gly Gly Pro Asn Arg Tyr Ser Ser Gly Asp
 50 55 60
 Ala Phe Ser Pro Thr Ala Ala Ile Pro Pro Pro Met Met Asn Pro Asn
 65 70 75 80
 40Asp Leu Pro Leu Gly Ala Ala Glu Thr Met Met Pro Leu Glu Pro Arg

112

| | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | | 85 | | | | | 90 | | | | 95 | | | |
| | Asp | Leu | Pro | Phe | Asp | Val | Tyr | Asp | Pro | His | Asn | Pro | Asn | Val | Lys | Met |
| | | | | 100 | | | | | 105 | | | | 110 | | | |
| | Ser | Lys | Phe | Asp | Asn | Ile | Gly | Ala | Val | Leu | Arg | His | Arg | Ser | Arg | Thr |
| 5 | | | 115 | | | | | 120 | | | | | 125 | | | |
| | Gln | Pro | Arg | Thr | Thr | Ala | Phe | Trp | Val | Leu | Asp | Ala | Lys | Gly | Lys | Glu |
| | | | 130 | | | | | 135 | | | | | 140 | | | |
| | Thr | Ala | Ser | Ile | Thr | Trp | Glu | Lys | Val | Ala | Ser | Arg | Ala | Glu | Lys | Val |
| | 145 | | | | | 150 | | | | | 155 | | | | 160 | |
| 10 | Ala | Lys | Val | Ile | Arg | Asp | Lys | Ser | Asn | Leu | Tyr | Arg | Gly | Asp | Arg | Val |
| | | | | 165 | | | | | | 170 | | | | 175 | | |
| | Ala | Leu | Val | Tyr | Arg | Asp | Thr | Glu | Ile | Ile | Asp | Phe | Val | Val | Ala | Leu |
| | | | | 180 | | | | | 185 | | | | | 190 | | |
| | Met | Gly | Cys | Phe | Ile | Ala | Gly | Val | Val | Ala | Val | Pro | Ile | Asn | Ser | Val |
| 15 | | | 195 | | | | | 200 | | | | | 205 | | | |
| | Asp | Asp | Tyr | Gln | Lys | Leu | Ile | Leu | Leu | Leu | Thr | Thr | Thr | Gln | Ala | His |
| | | | 210 | | | | | 215 | | | | | 220 | | | |
| | Leu | Ala | Leu | Thr | Thr | Asp | Asn | Asn | Leu | Lys | Ala | Phe | His | Arg | Asp | Ile |
| | | | 225 | | | 230 | | | | 235 | | | | | 240 | |
| 20 | Ser | Gln | Asn | Arg | Leu | Lys | Trp | Pro | Ser | Gly | Val | Glu | Trp | Trp | Lys | Thr |
| | | | | 245 | | | | | | 250 | | | | | 255 | |
| | Asn | Glu | Phe | Gly | Ser | His | His | Pro | Lys | Lys | His | Asp | Asp | Thr | Pro | Ala |
| | | | 260 | | | | | | 265 | | | | | 270 | | |
| | Leu | Gln | Val | Pro | Glu | Val | Ala | Tyr | Ile | Glu | Phe | Ser | Arg | Ala | Pro | Thr |
| 25 | | | 275 | | | | | 280 | | | | | | 285 | | |
| | Gly | Asp | Leu | Arg | Gly | Val | Val | Leu | Ser | His | Arg | Thr | Ile | Met | His | Gln |
| | | | 290 | | | | | 295 | | | | | 300 | | | |
| | Met | Ala | Cys | Ile | Ser | Ala | Met | Ile | Ser | Thr | Ile | Pro | Thr | Asn | Ala | Gln |
| | | | 305 | | | 310 | | | | | 315 | | | | 320 | |
| 30 | Ser | Gln | Asp | Thr | Phe | Ser | Thr | Ser | Leu | Arg | Asp | Ala | Glu | Gly | Lys | Phe |
| | | | | 325 | | | | | | 330 | | | | | 335 | |
| | Val | Ala | Pro | Ala | Pro | Ser | Arg | Asn | Pro | Thr | Glu | Val | Ile | Leu | Thr | Tyr |
| | | | 340 | | | | | | 345 | | | | | 350 | | |
| | Leu | Asp | Pro | Arg | Glu | Ser | Ala | Gly | Leu | Ile | Leu | Ser | Val | Leu | Phe | Ala |
| 35 | | | 355 | | | | | 360 | | | | | 365 | | | |
| | Val | Tyr | Gly | Gly | His | Thr | Thr | Val | Trp | Leu | Glu | Thr | Ala | Thr | Met | Glu |
| | | | 370 | | | | 375 | | | | | | 380 | | | |
| | Thr | Pro | Gly | Leu | Tyr | Ala | His | Leu | Ile | Thr | Lys | Tyr | Lys | Ser | Asn | Ile |
| | | | 385 | | | 390 | | | | | 395 | | | | 400 | |
| 40 | Leu | Leu | Ala | Asp | Tyr | Pro | Gly | Leu | Lys | Arg | Ala | Ala | Tyr | Asn | Tyr | Gln |

113

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | | | | 405 | | | | | 410 | | | | | 415 | | | |
| | Gln | Asp | Pro | Met | Ala | Thr | Arg | Asn | Phe | Lys | Lys | Asn | Thr | Glu | Pro | Asn | |
| | | | | 420 | | | | | 425 | | | | | 430 | | | |
| | Phe | Ala | Ser | Val | Lys | Ile | Cys | Leu | Ile | Asp | Thr | Leu | Thr | Val | Asp | Cys | |
| 5 | | | 435 | | | | | 440 | | | | | | 445 | | | |
| | Glu | Phe | His | Glu | Ile | Leu | Gly | Asp | Arg | Tyr | Phe | Arg | Pro | Leu | Arg | Asn | |
| | | | 450 | | | | 455 | | | | | 460 | | | | | |
| | Pro | Arg | Ala | Arg | Glu | Leu | Ile | Ala | Pro | Met | Leu | Cys | Leu | Pro | Glu | His | |
| | 465 | | | | | 470 | | | | | 475 | | | | 480 | | |
| 10 | Gly | Gly | Met | Ile | Ile | Ser | Val | Arg | Asp | Trp | Leu | Gly | Gly | Glu | Glu | Arg | |
| | | | | 485 | | | | | 490 | | | | | | 495 | | |
| | Met | Gly | Cys | Pro | Leu | Ser | Ile | Ala | Val | Glu | Glu | Ser | Asp | Asn | Asp | Glu | |
| | | | 500 | | | | | 505 | | | | | | 510 | | | |
| | Asp | Asp | Thr | Glu | Asp | Lys | Tyr | Ala | Ala | Ala | Asn | Gly | Tyr | Ser | Ser | Leu | |
| 15 | | | 515 | | | | | 520 | | | | | 525 | | | | |
| | Ile | Gly | Gly | Gly | Thr | Thr | Lys | Asn | Lys | Lys | Glu | Lys | Lys | Lys | Lys | Gly | |
| | | | 530 | | | | 535 | | | | | 540 | | | | | |
| | Pro | Thr | Glu | Leu | Thr | Glu | Ile | Leu | Leu | Asp | Lys | Glu | Ala | Leu | Lys | Met | |
| | 545 | | | | | 550 | | | | 555 | | | | | 560 | | |
| 20 | Asn | Glu | Val | Ile | Val | Leu | Ala | Ile | Gly | Glu | Glu | Ala | Ser | Lys | Arg | Ala | |
| | | | | 565 | | | | | 570 | | | | | 575 | | | |
| | Asn | Glu | Pro | Gly | Thr | Met | Arg | Val | Gly | Ala | Phe | Gly | Tyr | Pro | Ile | Pro | |
| | | | 580 | | | | | 585 | | | | | 590 | | | | |
| | Asp | Ala | Thr | Leu | Ala | Ile | Val | Asp | Pro | Glu | Thr | Ser | Leu | Leu | Cys | Ser | |
| 25 | | | 595 | | | | | 600 | | | | | 605 | | | | |
| | Pro | Tyr | Ser | Ile | Gly | Glu | Ile | Trp | Val | Asp | Ser | Pro | Ser | Leu | Ser | Gly | |
| | | | 610 | | | | 615 | | | | | 620 | | | | | |
| | Gly | Phe | Trp | Gln | Leu | Gln | Lys | His | Thr | Glu | Thr | Ile | Phe | His | Ala | Arg | |
| | 625 | | | | 630 | | | | | 635 | | | | | 640 | | |
| 30 | Pro | Tyr | Arg | Phe | Val | Glu | Gly | Ser | Pro | Thr | Pro | Gln | Leu | Leu | Glu | Leu | |
| | | | | 645 | | | | | 650 | | | | 655 | | | | |
| | Glu | Phe | Leu | Arg | Thr | Gly | Leu | Leu | Gly | Phe | Val | Val | Glu | Gly | Lys | Ile | |
| | | | 660 | | | | 665 | | | | | 670 | | | | | |
| | Phe | Val | Leu | Gly | Leu | Tyr | Glu | Asp | Arg | Ile | Arg | Gln | Arg | Val | Glu | Trp | |
| 35 | | | 675 | | | | 680 | | | | | 685 | | | | | |
| | Val | Glu | Asn | Gly | Gln | Leu | Glu | Ala | Glu | His | Arg | Tyr | Phe | Phe | Val | Gln | |
| | | | 690 | | | | 695 | | | | | 700 | | | | | |
| | His | Leu | Val | Thr | Ser | Ile | Met | Lys | Ala | Val | Pro | Lys | Ile | Tyr | Asp | Cys | |
| | 705 | | | | 710 | | | | 715 | | | | | 720 | | | |
| 40 | Ser | Ser | Phe | Asp | Ser | Tyr | Val | Asn | Gly | Glu | Tyr | Leu | Pro | Ile | Ile | Leu | |

735

| | | | | | | | | | | | | | | | | |
|------|-----|-----|------|-----|-----|------|-----|------|-----|-----|------|------|------|-----|-----|------|
| Ile | Glu | Thr | Gln | Ala | Ala | Ser | Thr | Ala | Pro | Thr | Asn | Pro | Gly | Gly | Pro | |
| | | | | 740 | | | | | 745 | | | | | 750 | | |
| Pro | Gln | Gln | Leu | Asp | Ile | Pro | Phe | Leu | Asp | Ser | Leu | Ser | Glu | Arg | Cys | |
| 5 | | | 755 | | | | | 760 | | | | | 765 | | | |
| Met | Glu | Val | Leu | Tyr | Gln | Glu | His | His | Leu | Arg | Val | Tyr | Cys | Val | Met | |
| | | | 770 | | | | 775 | | | | | | 780 | | | |
| Ile | Thr | Ala | Pro | Asn | Thr | Leu | Pro | Arg | Val | Ile | Lys | Asn | Gly | Arg | Arg | |
| 785 | | | | | | 790 | | | | | 795 | | | | | 800 |
| 10 | Glu | Ile | Gly | Asn | Met | Leu | Cys | Arg | Arg | Glu | Phe | Asp | Asn | Gly | Ser | Leu |
| | | | | | 805 | | | | | | 810 | | | | 815 | |
| Pro | Cys | Val | His | Val | Lys | Phe | Gly | Ile | Glu | Arg | Ser | Val | Gln | Asn | Ile | |
| | | | | | 820 | | | | 825 | | | | | 830 | | |
| Ala | Leu | Gly | Asp | Asp | Pro | Ala | Gly | Gly | Met | Trp | Ser | Phe | Glu | Ala | Ser | |
| 15 | | | 835 | | | | | 840 | | | | | 845 | | | |
| Met | Ala | Arg | Gln | Gln | Phe | Leu | Met | Leu | Gln | Asp | Lys | Gln | Tyr | Ser | Gly | |
| | | | | | | 855 | | | | | | 860 | | | | |
| Val | Asp | His | Arg | Glu | Val | Val | Ile | Asp | Asp | Arg | Thr | Ser | Thr | Pro | Leu | |
| 865 | | | | | | 870 | | | | | 875 | | | | | 880 |
| 20 | Asn | Gln | Phe | Ser | Asn | Ile | His | Asp | Leu | Met | Gln | Trp | Arg | Val | Ser | Arg |
| | | | | | 885 | | | | | | 890 | | | | 895 | |
| Gln | Ala | Glu | Glu | Leu | Ala | Tyr | Cys | Thr | Val | Asp | Gly | Arg | Gly | Lys | Glu | |
| | | | | | 900 | | | | 905 | | | | | 910 | | |
| Gly | Lys | Gly | Val | Asn | Trp | Lys | Lys | Phe | Asp | Gln | Lys | Val | Ala | Gly | Val | |
| 25 | | | 915 | | | | | 920 | | | | | 925 | | | |
| Ala | Met | Tyr | Leu | Lys | Asn | Lys | Val | Lys | Val | Gln | Ala | Gly | Asp | His | Leu | |
| | | | | | | 935 | | | | | 940 | | | | | |
| Leu | Leu | Met | Tyr | Thr | His | Ser | Glu | Glu | Phe | Val | Tyr | Ala | Val | His | Ala | |
| 945 | | | | | | 950 | | | | | 955 | | | | | 960 |
| 30 | Cys | Phe | Val | Leu | Gly | Ala | Val | Cys | Ile | Pro | Met | Ala | Pro | Ile | Asp | Gln |
| | | | | | 965 | | | | | 970 | | | | | 975 | |
| Asn | Arg | Leu | Asn | Glu | Asp | Ala | Pro | Ala | Leu | Leu | His | Ile | Leu | Ala | Asp | |
| | | | | 980 | | | | | 985 | | | | 990 | | | |
| Phe | Lys | Val | Lys | Ala | Ile | Leu | Val | Asn | Ala | Asp | Val | Asp | His | Leu | Met | |
| 35 | | | 995 | | | | | 1000 | | | | | 1005 | | | |
| Lys | Ile | Lys | Gln | Val | Ser | Gln | His | Ile | Lys | Gln | Ser | Ala | Ala | Ile | Leu | |
| | | | 1010 | | | | | 1015 | | | | 1020 | | | | |
| Lys | Ile | Ser | Val | Pro | Asn | Thr | Tyr | Ser | Thr | Thr | Lys | Pro | Pro | Lys | Gln | |
| 1025 | | | | | | 1030 | | | | | 1035 | | | | | 1040 |
| 40 | Ser | Ser | Gly | Cys | Arg | Asp | Leu | Lys | Leu | Thr | Ile | Arg | Pro | Ala | Trp | Ile |

115

1045 1050 1055

Gln Ala Gly Phe Pro Val Leu Val Trp Thr Tyr Trp Thr Pro Asp Gln

1060 1065 1070

Arg Arg Ile Ala Val Gln Leu Gly His Ser Gln Ile Met Ala Leu Cys

5 1075 1080 1085

Lys Val Gln Lys Glu Thr Cys Gln Met Thr Ser Thr Arg Pro Val Leu

1090 1095 1100

Gly Cys Val Arg Ser Thr Ile Gly Leu Gly Phe Leu His Thr Cys Leu

1105 1110 1115 1120

10Met Gly Ile Phe Leu Ala Ala Pro Thr Tyr Leu Val Ser Pro Val Asp

1125 1130 1135

Phe Ala Gln Asn Pro Asn Ile Leu Phe Gln Thr Leu Ser Arg Tyr Lys

1140 1145 1150

Ile Lys Asp Ala Tyr Ala Thr Ser Gln Met Leu Asp His Ala Ile Ala

15 1155 1160 1165

Arg Gly Ala Gly Lys Ser Met Ala Leu His Glu Leu Lys Asn Leu Met

1170 1175 1180

Ile Ala Thr Asp Gly Arg Pro Arg Val Asp Val Tyr Gln Arg Val Arg

1185 1190 1195 1200

20Val His Phe Ala Pro Ala Asn Leu Asp Pro Thr Ala Ile Asn Thr Val

1205 1210 1215

Tyr Ser His Val Leu Asn Pro Met Val Ala Ser Arg Ser Tyr Met Cys

1220 1225 1230

Ile Glu Pro Val Glu Leu His Leu Asp Val His Ala Leu Arg Arg Gly

25 1235 1240 1245

Leu Val Met Pro Val Asp Pro Asp Thr Glu Pro Asn Ala Leu Leu Val

1250 1255 1260

Gln Asp Ser Gly Met Val Pro Val Ser Thr Gln Ile Ser Ile Val Asn

1265 1270 1275 1280

30Pro Glu Thr Asn Gln Leu Cys Leu Asn Gly Glu Tyr Gly Glu Ile Trp

1285 1290 1295

Val Gln Ser Glu Ala Asn Ala Tyr Ser Phe Tyr Met Ser Lys Glu Arg

1300 1305 1310

Leu Asp Ala Glu Arg Phe Asn Gly Arg Thr Ile Asp Gly Asp Pro Asn

35 1315 1320 1325

Val Arg Tyr Val Arg Thr Gly Asp Leu Gly Phe Leu His Ser Val Thr

1330 1335 1340

Arg Pro Ile Gly Pro Asn Gly Ala Pro Val Asp Met Gln Val Leu Phe

1345 1350 1355 1360

40Val Leu Gly Ser Ile Gly Asp Thr Phe Glu Val Asn Gly Leu Asn His

116

| | | | |
|----|---|------|------|
| | 1365 | 1370 | 1375 |
| | Phe Ser Met Asp Ile Glu Gln Ser Val Glu Arg Cys His Arg Asn Ile | | |
| | 1380 | 1385 | 1390 |
| | Val Pro Gly Gly Cys Ala Val Phe Gln Ala Gly Gly Leu Val Val Val | | |
| 5 | 1395 | 1400 | 1405 |
| | Val Val Glu Ile Phe Arg Arg Asn Phe Leu Ala Ser Met Val Pro Val | | |
| | 1410 | 1415 | 1420 |
| | Ile Val Asn Ala Ile Leu Asn Glu His Gln Leu Val Ile Asp Ile Val | | |
| | 1425 | 1430 | 1435 |
| 10 | Ser Phe Val Gln Lys Gly Asp Phe His Arg Ser Arg Leu Gly Glu Lys | | |
| | 1445 | 1450 | 1455 |
| | Gln Arg Gly Lys Ile Leu Ala Gly Trp Val Thr Arg Lys Met Arg Thr | | |
| | 1460 | 1465 | 1470 |
| | Ile Ala Gln Tyr Ser Ile Arg Asp Pro Asn Gly Gln Asp Ser Gln Met | | |
| 15 | 1475 | 1480 | 1485 |
| | Met Ile Thr Glu Glu Pro Gly Pro Arg Ala Ser Met Thr Gly Ser Met | | |
| | 1490 | 1495 | 1500 |
| | Leu Gly Arg Met Gly Gly Pro Ala Ser Ile Lys Ala Gly Ser Thr Arg | | |
| | 1505 | 1510 | 1515 |
| 20 | Ala Pro Ser Leu Met Gly Met Thr Ala Thr Met Asn Asn Leu Ser Leu | | |
| | 1525 | 1530 | 1535 |
| | Thr Gln Gln Gln Gln Gln Tyr Gln Gln Pro Gly Met Tyr Ala Gln | | |
| | 1540 | 1545 | 1550 |
| | Gln Gln Gly Met His Pro Gln Gln Gln His Gln Phe Ser Met Ser Asn | | |
| 25 | 1555 | 1560 | 1565 |
| | Thr Pro Pro Gln Gly Pro Pro Gln Gly Val Glu Leu His Asp Pro Ser | | |
| | 1570 | 1575 | 1580 |
| | Asp Arg Thr Pro Thr Asp Asn Arg His Ser Phe Leu Ala Asp Pro Arg | | |
| | 1585 | 1590 | 1595 |
| 30 | Met Gln Asn Gln Gly Gln Met Asn Glu Thr Gly Ala Tyr Glu Pro Met | | |
| | 1605 | 1610 | 1615 |
| | Asn Tyr Gln Asn Ala Tyr His Pro His Gln Gln Gln Tyr Glu Ser Glu | | |
| | 1620 | 1625 | 1630 |
| | Asp Gly Gly Ser Arg Leu Ser Gly Pro Val Pro Asp Val Leu Arg Pro | | |
| 35 | 1635 | 1640 | 1645 |
| | Gly Pro Ser Ser Gly Ser Ile Glu Gln His Asp Gln Ala Asn Asn Asp | | |
| | 1650 | 1655 | 1660 |
| | Asn Asn Met Trp Asn Asn Arg Glu Tyr Tyr Gly Asn Ser Pro Ser Tyr | | |
| | 1665 | 1670 | 1675 |
| 40 | Ala Gly Gly Tyr Thr Gln Asp Gly Asn Ile His Glu Gln Gln Gln His | | |

117

1685 1690 1695
 Asp Glu Tyr Thr Ser Asn Ala Ser Tyr Gly Gly Asn Gln Gly Ala Gly
 1700 1705 1710
 Gly Gly Ser Gly Gly Gly Gly Gly Leu Arg Val Ala Asn Arg Asp Ser
 5 1715 1720 1725
 Ser Asp Ser Glu Gly Ala Asp Asp Asp Ala Trp Arg Arg Asp Ala Leu
 1730 1735 1740
 Ala Gln Ile Asn Phe Ala Gly Gly Ala Ala Ala Ala Ser Ala Gly Ala
 1745 1750 1755 1760
 10Pro Ala Ala Gly Ala Ser Ser Ser Gln Pro Gly His Ala Gln
 1765 1770

<210> 186

<211> 530

15<212> PRT

<213> Cochliobolus heterostrophus

<400> 186

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 20 25 30
 Ser Lys Arg Ala Asn Glu Pro Gly Thr Met Arg Val Gly Ala Phe Gly
 35 40 45
 25Tyr Pro Ile Pro Asp Ala Thr Leu Ala Ile Val Asp Pro Glu Thr Ser
 50 55 60
 Leu Leu Cys Ser Pro Tyr Ser Ile Gly Glu Ile Trp Val Asp Ser Pro
 65 70 75 80
 Ser Leu Ser Gly Gly Phe Trp Gln Leu Gln Lys His Thr Glu Thr Ile
 30 85 90 95
 Phe His Ala Arg Pro Tyr Arg Phe Val Glu Gly Ser Pro Thr Pro Gln
 100 105 110
 Leu Leu Glu Leu Glu Phe Leu Arg Thr Gly Leu Leu Gly Phe Val Val
 115 120 125
 35Glu Gly Lys Ile Phe Val Leu Gly Leu Tyr Glu Asp Arg Ile Arg Gln
 130 135 140
 Arg Val Glu Trp Val Glu Asn Gly Gln Leu Glu Ala Glu His Arg Tyr
 145 150 155 160
 Phe Phe Val Gln His Leu Val Thr Ser Ile Met Lys Ala Val Pro Lys
 40 165 170 175

118

| | | | |
|---|-----|-----|-----|
| Ile Tyr Asp Cys Ser Ser Phe Asp Ser Tyr Val Asn Gly Glu Tyr Leu | | | |
| 180 | 185 | 190 | |
| Pro Ile Ile Leu Ile Glu Thr Gln Ala Ala Ser Thr Ala Pro Thr Asn | | | |
| 195 | 200 | 205 | |
| 5Pro Gly Gly Pro Pro Gln Gln Leu Asp Ile Pro Phe Leu Asp Ser Leu | | | |
| 210 | 215 | 220 | |
| Ser Glu Arg Cys Met Glu Val Leu Tyr Gln Glu His His Leu Arg Val | | | |
| 225 | 230 | 235 | 240 |
| Tyr Cys Val Met Ile Thr Ala Pro Asn Thr Leu Pro Arg Val Ile Lys | | | |
| 10 | 245 | 250 | 255 |
| Asn Gly Arg Arg Glu Ile Gly Asn Met Leu Cys Arg Arg Glu Phe Asp | | | |
| 260 | 265 | 270 | |
| Asn Gly Ser Leu Pro Cys Val His Val Lys Phe Gly Ile Glu Arg Ser | | | |
| 275 | 280 | 285 | |
| 15Val Gln Asn Ile Ala Leu Gly Asp Asp Pro Ala Gly Gly Met Trp Ser | | | |
| 290 | 295 | 300 | |
| Phe Glu Ala Ser Met Ala Arg Gln Gln Phe Leu Met Leu Gln Asp Lys | | | |
| 305 | 310 | 315 | 320 |
| Gln Tyr Ser Gly Val Asp His Arg Glu Val Val Ile Asp Asp Arg Thr | | | |
| 20 | 325 | 330 | 335 |
| Ser Thr Pro Leu Asn Gln Phe Ser Asn Ile His Asp Leu Met Gln Trp | | | |
| 340 | 345 | 350 | |
| Arg Val Ser Arg Gln Ala Glu Glu Leu Ala Tyr Cys Thr Val Asp Gly | | | |
| 355 | 360 | 365 | |
| 25Arg Gly Lys Glu Gly Lys Gly Val Asn Trp Lys Lys Phe Asp Gln Lys | | | |
| 370 | 375 | 380 | |
| Val Ala Gly Val Ala Met Tyr Leu Lys Asn Lys Val Lys Val Gln Ala | | | |
| 385 | 390 | 395 | 400 |
| Gly Asp His Leu Leu Leu Met Tyr Thr His Ser Glu Glu Phe Val Tyr | | | |
| 30 | 405 | 410 | 415 |
| Ala Val His Ala Cys Phe Val Leu Gly Ala Val Cys Ile Pro Met Ala | | | |
| 420 | 425 | 430 | |
| Pro Ile Asp Gln Asn Arg Leu Asn Glu Asp Ala Pro Ala Leu Leu His | | | |
| 435 | 440 | 445 | |
| 35Ile Leu Ala Asp Phe Lys Val Lys Ala Ile Leu Val Asn Ala Asp Val | | | |
| 450 | 455 | 460 | |
| Asp His Leu Met Lys Ile Lys Gln Val Ser Gln His Ile Lys Gln Ser | | | |
| 465 | 470 | 475 | 480 |
| Ala Ala Ile Leu Lys Ile Ser Val Pro Asn Thr Tyr Ser Thr Thr Lys | | | |
| 40 | 485 | 490 | 495 |

119

Pro Pro Lys Gln Ser Ser Gly Cys Arg Asp Leu Lys Leu Thr Ile Arg
 500 505 510
 Pro Ala Trp Ile Gln Ala Gly Phe Pro Val Leu Val Trp Thr Tyr Trp
 515 520 525
 5Thr Pro
 530

<210> 187
 <211> 1767
 10<212> DNA
 <213> *Cochliobolus heterostrophus*

<400> 187

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| 15tcattctgtgt | acctcataaa | agtctcgccc | ccagaacacc | ggagcaaacg | acaaggatat | 120 |
| gagactgcct | gtaatcatgg | tccagaatca | agaggatgct | ggattgacga | cttcaacatc | 180 |
| gacaccgaca | tggatgttga | atggccagat | actggaaaga | cagtcaagta | tcacctgacc | 240 |
| atcaccaata | ccactggagc | tccagacggt | tttgaaaggc | cgatgttctt | gattaatggc | 300 |
| caatacccag | gaccaactat | tactgccgac | tggggagatg | ttctagagat | cacagttacc | 360 |
| 20aatggccttg | aaaacaacgg | tacaggtata | cattggcacg | gtctgaggca | actcgggaca | 420 |
| aacgaacaag | atggcgtaaa | tgggtatcact | gaatgcccaa | tcgcacccgg | tgactccaag | 480 |
| ctctacagat | tcaaagcaac | tcaatatggc | actacctggt | atcactcgca | ctactcgggtg | 540 |
| cagtatggtg | acggcatcgt | gggtcctctg | atcatcaaag | gacctcaac | ggcgaactac | 600 |
| gatattgatc | ttggcgcttt | cccaatgact | gactgggttc | acgcaaccac | cttcaccgtc | 660 |
| 25aacgctgcag | ccgttcatgc | aaatggccct | ccaactgctg | acaatgtcct | tgtcaatggc | 720 |
| tccatgacct | catcttttgg | cggcaagtac | gccgaaacga | tcctaactcc | gggaaaatct | 780 |
| cacttgctgc | gtttgatgaa | cgttggtatt | aacaactacc | ttcatgtcgg | cctcgatggg | 840 |
| catcagttcc | aggtcatttc | ggctgatttc | acgcccattg | aacctttcta | cacggacagc | 900 |
| ttggtccttg | cagtcgggtca | acggtatgaa | gtcatcatca | acgcaactga | agctgtgggc | 960 |
| 30aactactggc | tacgtgttgg | taccggcggt | aactgcgacg | gtcccaatgc | caatgcagca | 1020 |
| aatatcagga | gtatcttccg | atatgctggc | gctccaactg | aagacccaga | cacgactgggt | 1080 |
| tcgcttccgt | cgggctgcta | cgatgaggat | gttgtaacct | atgccaaagac | gactgttcct | 1140 |
| caggagatgc | ccgaacagtt | gagcgtgggc | ttcaacccta | actggactag | tgacgtgacg | 1200 |
| caaaatcagg | gtctggtcca | atggctcgtc | aacggtaatc | ccatggcagt | tgatcttgaa | 1260 |
| 35gtccctactc | tgcagtcgggt | gttggatggc | aatgttacct | acggaaaaca | ccgccacgtg | 1320 |
| tttgcagtcg | acgagaaaca | ccaatggcaa | tattgggtca | tccaacaaaa | cagttctaac | 1380 |
| ccaccacttc | ctcaccat | ccacctccac | ggccacgact | tctacgtcct | cgcacagggtc | 1440 |
| gaaaacgcag | tctggaacgg | agatatttca | accctgaaga | cggacaaccc | catccgtcgg | 1500 |
| gacacggccg | atcttcccgc | tggaggctac | ttggtccttg | ctttcgagtc | ggacaaccct | 1560 |
| 40ggcgcacatggc | ttatgcactg | ccacatcccc | ttccacgttg | ctgccggtct | cgggtgtccag | 1620 |

120

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ttcctcgagc gcgaatccga aatcaaggcc caagatggat acgcagagat gcacaggaca 1680
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5<210> 188

<211> 588

<212> PRT

<213> *Cochliobolus heterostrophus*

10<400> 188

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Pro Thr Val Thr Ser Ser Val Tyr Leu Ile Lys Val Ser Pro Pro Glu
      20              25              30
15His Arg Ser Lys Arg Gln Gly Tyr Glu Thr Ala Cys Asn His Gly Pro
      35              40              45
Glu Ser Arg Gly Cys Trp Ile Asp Asp Phe Asn Ile Asp Thr Asp Met
      50              55              60
Asp Val Glu Trp Pro Asp Thr Gly Lys Thr Val Lys Tyr His Leu Thr
2065              70              75              80
Ile Thr Asn Thr Thr Gly Ala Pro Asp Gly Phe Glu Arg Pro Met Phe
      85              90              95
Leu Ile Asn Gly Gln Tyr Pro Gly Pro Thr Ile Thr Ala Asp Trp Gly
      100             105             110
25Asp Val Leu Glu Ile Thr Val Thr Asn Gly Leu Glu Asn Asn Gly Thr
      115             120             125
Gly Ile His Trp His Gly Leu Arg Gln Leu Gly Thr Asn Glu Gln Asp
      130             135             140
Gly Val Asn Gly Ile Thr Glu Cys Pro Ile Ala Pro Gly Asp Ser Lys
30145             150             155             160
Leu Tyr Arg Phe Lys Ala Thr Gln Tyr Gly Thr Thr Trp Tyr His Ser
      165             170             175
His Tyr Ser Val Gln Tyr Gly Asp Gly Ile Val Gly Pro Leu Ile Ile
      180             185             190
35Lys Gly Pro Ser Thr Ala Asn Tyr Asp Ile Asp Leu Gly Ala Phe Pro
      195             200             205
Met Thr Asp Trp Phe His Ala Thr Thr Phe Thr Val Asn Ala Ala Ala
      210             215             220
Val His Ala Asn Gly Pro Pro Thr Ala Asp Asn Val Leu Val Asn Gly
40225             230             235             240

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121

| | | | |
|---|-----|-----|-----|
| Ser Met Thr Ser Ser Phe Gly Gly Lys Tyr Ala Glu Thr Ile Leu Thr | | | |
| | 245 | 250 | 255 |
| Pro Gly Lys Ser His Leu Leu Arg Leu Met Asn Val Gly Ile Asn Asn | | | |
| | 260 | 265 | 270 |
| 5Tyr Leu His Val Gly Leu Asp Gly His Gln Phe Gln Val Ile Ser Ala | | | |
| | 275 | 280 | 285 |
| Asp Phe Thr Pro Ile Glu Pro Phe Tyr Thr Asp Ser Leu Val Leu Ala | | | |
| | 290 | 295 | 300 |
| Val Gly Gln Arg Tyr Glu Val Ile Ile Asn Ala Thr Glu Ala Val Gly | | | |
| 10305 | 310 | 315 | 320 |
| Asn Tyr Trp Leu Arg Val Gly Thr Gly Gly Asn Cys Asp Gly Pro Asn | | | |
| | 325 | 330 | 335 |
| Ala Asn Ala Ala Asn Ile Arg Ser Ile Phe Arg Tyr Ala Gly Ala Pro | | | |
| | 340 | 345 | 350 |
| 15Thr Glu Asp Pro Asp Thr Thr Gly Ser Leu Pro Ser Gly Cys Tyr Asp | | | |
| | 355 | 360 | 365 |
| Glu Asp Val Val Pro Tyr Ala Lys Thr Thr Val Pro Gln Glu Met Pro | | | |
| | 370 | 375 | 380 |
| Glu Gln Leu Ser Val Gly Phe Asn Pro Asn Trp Thr Ser Asp Val Thr | | | |
| 20385 | 390 | 395 | 400 |
| Gln Asn Gln Gly Leu Val Gln Trp Leu Val Asn Gly Asn Pro Met Ala | | | |
| | 405 | 410 | 415 |
| Val Asp Leu Glu Val Pro Thr Leu Gln Ser Val Leu Asp Gly Asn Val | | | |
| | 420 | 425 | 430 |
| 25Thr Tyr Gly Asn Asn Arg His Val Phe Ala Val Asp Glu Lys His Gln | | | |
| | 435 | 440 | 445 |
| Trp Gln Tyr Trp Val Ile Gln Gln Asn Ser Ser Asn Pro Pro Leu Pro | | | |
| | 450 | 455 | 460 |
| His Pro Ile His Leu His Gly His Asp Phe Tyr Val Leu Ala Gln Val | | | |
| 30465 | 470 | 475 | 480 |
| Glu Asn Ala Val Trp Asn Gly Asp Ile Ser Thr Leu Lys Thr Asp Asn | | | |
| | 485 | 490 | 495 |
| Pro Ile Arg Arg Asp Thr Ala Asp Leu Pro Ala Gly Gly Tyr Leu Val | | | |
| | 500 | 505 | 510 |
| 35Leu Ala Phe Glu Ser Asp Asn Pro Gly Ala Trp Leu Met His Cys His | | | |
| | 515 | 520 | 525 |
| Ile Pro Phe His Val Ala Ala Gly Leu Gly Val Gln Phe Leu Glu Arg | | | |
| | 530 | 535 | 540 |
| Glu Ser Glu Ile Lys Ala Gln Asp Gly Tyr Ala Glu Met His Arg Thr | | | |
| 40545 | 550 | 555 | 560 |

122

Cys Ala Asn Trp Gln Ser Trp Arg Tyr Lys Tyr His Pro Asn Gly Ile

565

570

575

Leu Phe Pro Gly Asp Ser Gly Leu Arg Arg Arg Asn

580

585

5

<210> 189

<211> 327

<212> DNA

<213> Cochliobolus heterostrophus

10

<400> 189

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aacgaagagg aggaacaaag caaaggctcc ggcggcctct tgagcgcaat cggagatcca 120

gtcggtagct ctctttatcc ccccttcctc ctccatctct caaccacaa cctaaccat 180

15ctcccaaggc aacgtcctca acaccgccct ccgccccgtc ggcgcgccgc tcgagaaatt 240

cgtcacaggc ccgctgggcg aggggtctcg cggcaccaca cgcggcgcgc tgggcccgtt 300

gatgggccac gaggacgagc gctctga 327

<210> 190

20<211> 108

<212> PRT

<213> Cochliobolus heterostrophus

<400> 190

25Met Ala Gln Glu Lys Lys Glu Glu Gln Pro Gln Gln Asp His Ile Pro

1

5

10

15

Thr Ser Pro Gln Asn Glu Glu Glu Glu Gln Ser Lys Gly Ser Gly Gly

20

25

30

Leu Leu Ser Ala Ile Gly Asp Pro Val Gly Thr Ser Pro Tyr Pro Pro

30

35

40

45

Phe Leu Leu His Leu Ser Thr His Asn Leu Thr His Leu Pro Arg Gln

50

55

60

Arg Pro Gln His Arg Pro Pro Pro Arg Arg Arg Ala Ala Arg Glu Ile

65

70

75

80

35Arg His Arg Pro Ala Gly Arg Gly Ser Arg Arg His His Thr Arg Arg

85

90

95

Ala Gly Pro Val Asp Gly Pro Arg Gly Arg Ala Leu

100

105

40<210> 191

123

<211> 1626

<212> DNA

<213> Cochliobolus heterostrophus

5<400> 191

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| cggcgatctc | ttcctccaca | attgcgggct | gcgcgaactc | tgccaccacg | atggcggtt | 120 |
| acaggtcaat | tgcgctgcat | cagtgcgcgc | gcgcgcgaaa | ttgaccgctc | gaaatcaaag | 180 |
| cttttcaaag | atgcagacga | agcagttgca | gatgtacagc | ctggttccac | cgctctgagc | 240 |
| 10gcaggattcg | ggttgtgtgg | tgtcgcagac | actttgatcg | cagcgatgaa | gaagcgtggg | 300 |
| ccggagtcac | tacattcggt | aacagccgct | tcaaacaatg | ctggcattga | agacgttaga | 360 |
| ggattggcac | atcttataaa | gaacggacaa | gtcaagaagc | tcattataag | ttttctcggc | 420 |
| aacaacaagg | cgcttgagaa | gcagtatctg | agcgggtgga | ttgaaattga | gctttgtccg | 480 |
| caaggtacgc | ttgcagagag | gatacgcgct | ggcggcgagc | gcaccccagc | atctttacaca | 540 |
| 15cccactgcag | taaatacact | gttgcaagat | ggccagattc | cggccaagtt | tgacaaggag | 600 |
| ggcaaggctg | taggctacgg | acagaagcgt | gagggttagag | agttcaatgg | caagaagtcc | 660 |
| ctcatggaga | ctgcattgac | cggcgatgtc | gccattatcc | gtgcacacaa | ggccgatgaa | 720 |
| gctggtaact | gtgttttcag | atacaccacc | aaagcttttg | gacccatcat | ggccaaagcc | 780 |
| gcacgcctta | caattgtcga | agccgaagag | attgtcccta | taggcacctt | tgatgccaac | 840 |
| 20gaggtcgatc | tccctggcat | cttcgtcgac | cgcacgctcc | cagccaccgc | ccccagaagc | 900 |
| attgagatca | agaagctgcg | aaaaccggct | gcacccaaag | acgcctcatc | aaagaacgaa | 960 |
| gccgccgaac | gacgcgatcg | tatcgctcgt | cgggcagcaa | aggagctcaa | gcagggatac | 1020 |
| tacgtcaatc | tgggcgtcgg | catccccaca | gccgcagcag | cattcgtacc | cgatggcgctc | 1080 |
| aagggtgtggc | tgcaatccga | aaatggcatt | ctaggaatgg | gcccctaccc | gacggaagaa | 1140 |
| 25gaagtagacg | cagacattgt | caacgccggc | aaagaaaccg | taaccctcct | tcccggcgcc | 1200 |
| tcgacctttg | acagcgccga | atcctttggc | atgatccgcg | gcggccacgt | cgacgtatcc | 1260 |
| atccttggag | ctctacaagt | cagtgcctct | ggcgacctgg | ccaactacat | ggtgcccgcc | 1320 |
| aaagtcttca | agggatatgg | cggcgccatg | gatctcgtta | gcaatcccga | tgctacaaaa | 1380 |
| gtcgttgtcg | cgactgaaca | cgttgctaaa | gatggatcca | gcaagattgt | tcaggaatgc | 1440 |
| 30cagttgccgc | ttacaggagc | aaagtgcgtg | agcactatta | ttaccgatct | gtgtgtcttt | 1500 |
| gaagtaaaca | ggaagagggg | gactttgacg | ctgacggaga | cggcgccggg | ggttagcggt | 1560 |
| gaggatgtca | aggcgaagac | ggatgcgaag | tttgaagtcg | cgagtgatct | caagacgatg | 1620 |
| gagtag | | | | | | 1626 |

35<210> 192

<211> 541

<212> PRT

<213> Cochliobolus heterostrophus

40<400> 192

124

| | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Asp | Thr | Leu | Pro | Ala | Ser | Cys | Arg | Leu | Leu | Thr | Ala | Leu | Pro | Ser |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Arg | Leu | Cys | Ala | Arg | Arg | Ser | Leu | Pro | Pro | Gln | Leu | Arg | Ala | Ala | Arg |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| 5Thr | Leu | Pro | Pro | Arg | Trp | Arg | Leu | Thr | Gly | Gln | Leu | Arg | Cys | Ile | Ser |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Glu | Arg | Ala | Pro | Thr | Ile | Asp | Arg | Ser | Lys | Ser | Lys | Leu | Phe | Lys | Asp |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Ala | Asp | Glu | Ala | Val | Ala | Asp | Val | Gln | Pro | Gly | Ser | Thr | Val | Leu | Ser |
| 1065 | | | | | 70 | | | | | 75 | | | | 80 | |
| Ala | Gly | Phe | Gly | Leu | Cys | Gly | Val | Ala | Asp | Thr | Leu | Ile | Ala | Ala | Met |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Lys | Lys | Arg | Gly | Pro | Glu | Ser | Leu | His | Ser | Leu | Thr | Ala | Val | Ser | Asn |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| 15Asn | Ala | Gly | Ile | Glu | Asp | Val | Gly | Gly | Leu | Ala | His | Leu | Thr | Lys | Asn |
| | | | 115 | | | | | 120 | | | | | 125 | | |
| Gly | Gln | Val | Lys | Lys | Leu | Ile | Ile | Ser | Phe | Leu | Gly | Asn | Asn | Lys | Ala |
| | | 130 | | | | 135 | | | | | 140 | | | | |
| Leu | Glu | Lys | Gln | Tyr | Leu | Ser | Gly | Gly | Ile | Glu | Ile | Glu | Leu | Cys | Pro |
| 20145 | | | | | 150 | | | | | 155 | | | | 160 | |
| Gln | Gly | Thr | Leu | Ala | Glu | Arg | Ile | Arg | Ala | Gly | Gly | Ala | Gly | Ile | Pro |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Ala | Phe | Tyr | Thr | Pro | Thr | Ala | Val | Asn | Thr | Leu | Leu | Gln | Asp | Gly | Gln |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| 25Ile | Pro | Ala | Lys | Phe | Asp | Lys | Glu | Gly | Lys | Ala | Val | Gly | Tyr | Gly | Gln |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Lys | Arg | Glu | Val | Arg | Glu | Phe | Asn | Gly | Lys | Lys | Phe | Leu | Met | Glu | Thr |
| | | 210 | | | | 215 | | | | | 220 | | | | |
| Ala | Leu | Thr | Gly | Asp | Val | Ala | Ile | Ile | Arg | Ala | His | Lys | Ala | Asp | Glu |
| 30225 | | | | | 230 | | | | | 235 | | | | 240 | |
| Ala | Gly | Asn | Cys | Val | Phe | Arg | Tyr | Thr | Thr | Lys | Ala | Phe | Gly | Pro | Ile |
| | | | 245 | | | | | | 250 | | | | 255 | | |
| Met | Ala | Lys | Ala | Ala | Arg | Leu | Thr | Ile | Val | Glu | Ala | Glu | Glu | Ile | Val |
| | | 260 | | | | | | 265 | | | | | 270 | | |
| 35Pro | Ile | Gly | Thr | Phe | Asp | Ala | Asn | Glu | Val | Asp | Leu | Pro | Gly | Ile | Phe |
| | | 275 | | | | | 280 | | | | | | 285 | | |
| Val | Asp | Arg | Ile | Val | Pro | Ala | Thr | Ala | Pro | Lys | Asn | Ile | Glu | Ile | Lys |
| | | 290 | | | | 295 | | | | | 300 | | | | |
| Lys | Leu | Arg | Lys | Pro | Ala | Ala | Ser | Lys | Asp | Ala | Ser | Ser | Lys | Asn | Glu |
| 40305 | | | | | 310 | | | | | 315 | | | | 320 | |

| | | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ala | Ala | Glu | Arg | Arg | Asp | Arg | Ile | Ala | Arg | Arg | Ala | Ala | Lys | Glu | Leu | |
| | | | | 325 | | | | 330 | | | | 335 | | | | |
| Lys | Gln | Gly | Tyr | Tyr | Val | Asn | Leu | Gly | Val | Gly | Ile | Pro | Thr | Ala | Ala | |
| | | | | 340 | | | | 345 | | | | 350 | | | | |
| 5Ala | Ala | Phe | Val | Pro | Asp | Gly | Val | Lys | Val | Trp | Leu | Gln | Ser | Glu | Asn | |
| | | | | 355 | | | | 360 | | | | 365 | | | | |
| Gly | Ile | Leu | Gly | Met | Gly | Pro | Tyr | Pro | Thr | Glu | Glu | Glu | Val | Asp | Ala | |
| | | | | 370 | | | | 375 | | | | 380 | | | | |
| Asp | Ile | Val | Asn | Ala | Gly | Lys | Glu | Thr | Val | Thr | Leu | Leu | Pro | Gly | Ala | |
| 10385 | | | | | 390 | | | | 395 | | | | 400 | | | |
| Ser | Thr | Phe | Asp | Ser | Ala | Glu | Ser | Phe | Gly | Met | Ile | Arg | Gly | Gly | His | |
| | | | | 405 | | | | 410 | | | | 415 | | | | |
| Val | Asp | Val | Ser | Ile | Leu | Gly | Ala | Leu | Gln | Val | Ser | Ala | Ser | Gly | Asp | |
| | | | | 420 | | | | 425 | | | | 430 | | | | |
| 15Leu | Ala | Asn | Tyr | Met | Val | Pro | Gly | Lys | Val | Phe | Lys | Gly | Met | Gly | Gly | |
| | | | | 435 | | | | 440 | | | | 445 | | | | |
| Ala | Met | Asp | Leu | Val | Ser | Asn | Pro | Asp | Ala | Thr | Lys | Val | Val | Val | Ala | |
| | | | | 450 | | | | 455 | | | | 460 | | | | |
| Thr | Glu | His | Val | Ala | Lys | Asp | Gly | Ser | Ser | Lys | Ile | Val | Gln | Glu | Cys | |
| 20465 | | | | | 470 | | | | 475 | | | | 480 | | | |
| Gln | Leu | Pro | Leu | Thr | Gly | Ala | Lys | Cys | Val | Ser | Thr | Ile | Ile | Thr | Asp | |
| | | | | 485 | | | | 490 | | | | 495 | | | | |
| Leu | Cys | Val | Phe | Glu | Val | Asn | Arg | Lys | Arg | Gly | Thr | Leu | Thr | Leu | Thr | |
| | | | | 500 | | | | 505 | | | | 510 | | | | |
| 25Glu | Thr | Ala | Pro | Gly | Val | Ser | Val | Glu | Asp | Val | Lys | Ala | Lys | Thr | Asp | |
| | | | | 515 | | | | 520 | | | | 525 | | | | |
| Ala | Lys | Phe | Glu | Val | Ala | Ser | Asp | Leu | Lys | Thr | Met | Glu | | | | |
| | | | | 530 | | | | 535 | | | | 540 | | | | |

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40<400> 193

126

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ccgtacaaga ggaagccttg gccaaaacaa agggacatga aatataattcc tggcaaaagc      180
gagagcgatg gtggtggtgt caactgctgg tctgacagca acggagaccc tgactacgat      240
5gtcaggaaac tgctagactg gaacggcgat tggctacctg ctccggaatc atgggtccgct      300
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tcacaagagt gcaccagatc cgtatactac ccactcagta ctttcagtcc cgaagatgga      420
ccttgcaaaag agctggcacc tcgttactgg cttgaggcga aggttgaggg cagtaacttg      480
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gcgccggagg caagaatcga ccctaccgat gcagagcatc ctaccactca tctgatgctg      720
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gttggcattg gaacaaggat gcaaagtttt caaactaaca atattgacag gcgatttaca      960
actactacgt tgagcatacc atttacgcaa ccgagtttga tgggcgcact gaagatcaaa     1020
tccgccagcg aatcaacact gtcaccagtg caggccttcc atacttggtc gcagtctcaa     1080
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20

<210> 194

<211> 376

<212> PRT

<213> Cochliobolus heterostrophus

25

<220>

<221> SITE

<222> (1)...(376)

<223> Xaa = any amino acid

30

<400> 194

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Pro Arg Trp Asn Arg Gly Thr Gln Pro Tyr Lys Arg Lys Pro Trp Pro
          35             40             45
Lys Gln Arg Asp Met Lys Tyr Ile Pro Gly Lys Ser Glu Ser Asp Gly
          50             55             60
40Gly Gly Val Asn Cys Trp Ser Asp Ser Asn Gly Asp Pro Asp Tyr Asp

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127

| | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 65 | | 70 | | 75 | | 80 | | | | | | | | | |
| Val | Arg | Lys | Leu | Leu | Asp | Trp | Asn | Gly | Asp | Trp | Leu | Pro | Ala | Pro | Glu |
| | | | 85 | | | | | | 90 | | | | | 95 | |
| Ser | Trp | Ser | Ala | Arg | Arg | Gly | His | Glu | Asp | Arg | His | Leu | Gly | Ala | His |
| 5 | | | 100 | | | | | 105 | | | | | 110 | | |
| Val | Glu | Gln | Trp | Met | Asn | Gly | His | Ser | Gln | Glu | Cys | Thr | Arg | Ser | Val |
| | | | 115 | | | | | 120 | | | | | 125 | | |
| Tyr | Tyr | Pro | Leu | Ser | Thr | Phe | Ser | Pro | Glu | Asp | Gly | Pro | Cys | Lys | Glu |
| | 130 | | | | | | 135 | | | | 140 | | | | |
| 10Leu | Ala | Pro | Arg | Tyr | Trp | Leu | Glu | Ala | Lys | Val | Glu | Gly | Ser | Asn | Leu |
| | 145 | | | | | 150 | | | | 155 | | | | 160 | |
| Arg | Glu | Ser | Trp | Lys | Thr | Ile | Ser | Thr | Ser | Asp | Pro | Lys | Pro | Leu | Asp |
| | | | | 165 | | | | | | 170 | | | | 175 | |
| Asp | Thr | Asp | Ile | Thr | Ile | His | Pro | Pro | Trp | Trp | Glu | Leu | Tyr | Glu | Asp |
| 15 | | | 180 | | | | | 185 | | | | | 190 | | |
| Val | Val | Tyr | Ser | Glu | Val | Ile | His | Glu | Glu | Gly | Gln | Gly | Glu | Gln | His |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Phe | Lys | His | Arg | Ser | Cys | Tyr | Leu | Asn | Ser | Leu | Pro | Ala | Pro | Glu | Ala |
| | 210 | | | | | | 215 | | | | | 220 | | | |
| 20Arg | Ile | Asp | Pro | Thr | Asp | Ala | Glu | His | Pro | Thr | Thr | His | Leu | Met | Leu |
| | 225 | | | | | 230 | | | | 235 | | | | 240 | |
| Ala | Ser | Ala | Ala | Glu | Lys | Leu | Gln | Asp | Leu | Gln | Gln | Arg | Arg | Glu | Ala |
| | | | | 245 | | | | | | 250 | | | | 255 | |
| Lys | Glu | Arg | Arg | Leu | Leu | Ala | Lys | Arg | Asn | Arg | Pro | Val | Ala | Asn | Ser |
| 25 | | | 260 | | | | | 265 | | | | | 270 | | |
| Met | Phe | Pro | Met | Gln | Ala | Met | Glu | Asp | Arg | Arg | Leu | Arg | Pro | Lys | Thr |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Asn | Met | Tyr | Ile | Arg | Pro | Val | Gln | Pro | Ala | Asp | Val | Val | Gly | Ile | Gly |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| 30Thr | Arg | Met | Gln | Ser | Phe | Gln | Thr | Asn | Asn | Ile | Asp | Arg | Arg | Phe | Thr |
| | 305 | | | | | 310 | | | | 315 | | | | 320 | |
| Thr | Thr | Thr | Leu | Ser | Ile | Pro | Phe | Thr | Gln | Pro | Ser | Leu | Met | Gly | Ala |
| | | | | 325 | | | | | | 330 | | | | 335 | |
| Leu | Lys | Ile | Lys | Ser | Ala | Ser | Glu | Ser | Thr | Leu | Ser | Pro | Val | Gln | Ala |
| 35 | | | 340 | | | | | 345 | | | | | 350 | | |
| Phe | His | Thr | Trp | Ser | Gln | Ser | Gln | Arg | Ala | Thr | Ser | Pro | Gly | Pro | Ile |
| | | 355 | | | | | 360 | | | | | | 365 | | |
| Pro | Val | Met | Leu | Pro | Lys | Arg | Leu | | | | | | | | |
| | 370 | | | | | 375 | | | | | | | | | |

128

<210> 195

<211> 768

<212> DNA

<213> Cochliobolus heterostrophus

5

<400> 195

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ttcgaggtgg tccgtactat cgccctccca tccacccatg atttgcgctc gtcgaagatt      180
10acctggtcac ccctggtcac tccgcccttg acctcatcaa cacgcacatc ttcgcccacc      240
actacacctc cccgtcgatc atcacggaca ccacgtccct gctcgaatcg cgtcctcata      300
tccgacgacg acaccgcgcg cgtttacgat ctccgcgatg agaaatggaa tgccgtgatt      360
agcaatggct ctggtggcat ggggaagaat gttcacgctc agtttggagg aacagaggac      420
gaggtgcttg tttggaccga ctttaccgcc tgtgttaaga tatggtgctt gaagacgggt      480
15cgggtagtgg agatacgaga tccgaagttt cctggtaaag atggcaaggg gtgggggttac      540
cgacctgctg acgatactgg attgaggaat ggaaggggac aagggcgtgt tctggcatta      600
ttgtgtcgtg catcagggac cgatatcttg ttgcttcttg caccgcagac gtacaagggt      660
ctgaatcgag tcgaactccc tactacagac gccgctggtc tgagatggag tcgtgacggg      720
cgctggctgg ccactctgga cgctgcgtct gcggggttaca agctttga      768

```

20

<210> 196

<211> 255

<212> PRT

<213> Cochliobolus heterostrophus

25

<400> 196

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Met Glu Asn Met Glu Ile Ser Gln Gln Ile Lys Ser Thr Thr Leu Ser
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Val Pro Ser Pro Thr Ala Thr His Thr Ala Cys Val Asn Gly Ala Arg
30             20             25             30
Leu Gln Ile Arg Cys Leu Asn Thr Phe Glu Val Val Arg Thr Ile Ala
      35             40             45
Leu Pro Ser Thr His Asp Leu Arg Ser Ser Lys Ile Thr Trp Ser Pro
      50             55             60
35Leu Val Ile Pro Pro Leu Thr Ser Ser Thr Arg Thr Ser Ser Pro Thr
      65             70             75             80
Thr Thr Pro Pro Arg Arg Ser Ser Arg Thr Pro Arg Pro Cys Ser Asn
      85             90             95
Arg Val Leu Ile Ser Asp Asp Asp Thr Ala Arg Val Tyr Asp Leu Arg
40             100             105             110

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129

Asp Glu Lys Trp Asn Ala Val Ile Ser Asn Gly Ser Gly Gly Met Gly
 115 120 125
 Lys Asn Val His Val Glu Phe Gly Gly Thr Glu Asp Glu Val Leu Val
 130 135 140
 5Trp Thr Asp Phe Thr Ala Cys Val Lys Ile Trp Cys Leu Lys Thr Gly
 145 150 155 160
 Arg Val Val Glu Ile Arg Asp Pro Lys Phe Pro Gly Lys Asp Gly Lys
 165 170 175
 Gly Trp Gly Tyr Arg Pro Ala Asp Asp Thr Gly Leu Arg Asn Gly Arg
 10 180 185 190
 Gly Gln Gly Arg Val Leu Ala Leu Leu Cys Arg Ala Ser Gly Thr Asp
 195 200 205
 Ile Leu Leu Leu Leu Ala Pro Gln Thr Tyr Lys Val Leu Asn Arg Val
 210 215 220
 15Glu Leu Pro Thr Thr Asp Ala Ala Gly Leu Arg Trp Ser Arg Asp Gly
 225 230 235 240
 Arg Trp Leu Ala Ile Trp Asp Ala Ala Ser Ala Gly Tyr Lys Leu
 245 250 255

20<210> 197

<211> 723

<212> DNA

<213> *Cochliobolus heterostrophus*

25<400> 197

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 agacgattgg ggtcgcaggg gtgccagagg agtgctttca caggagcgta cattatgagg 180
 atcgagcggg gacggagact tcgcaggtcc caaatccaga ctgtgcaggg tgtgctgtcg 240
 30tctctgcttg cgcacattgt gccttcggag ttgaagctga gcatgccgat gccttgTTTT 300
 aggagcgcat ttctgttctt ttctagggcg gctttgggag gtgtggcggg ttgtggtgtg 360
 agtgtgaaac tacgggcgcc cagggtgtcg acttgctctg tgtacactgg tgcgctgggt 420
 acgtcgatga cgggtgtgtg gtcgaggaac aggatgggtg cgaatgtgcg ttagaaaagg 480
 atgcgaacac gacggtccca gccgccaact gcgagacgtt catgtccagg gaccattct 540
 35agactcttga tgcctaggcc ttctacatcc cattcgctga cgtcctcgga tgcttcgcgg 600
 gttatggtgc ggtacaaatg cccatccgcc gtatatatca aagcttgtaa cccgcagacg 660
 cagcgtccca gatggccagc cagcgcccg cagactcca tctcagacca gcggcgtctg 720
 tag 723

40<210> 198

130

<211> 240

<212> PRT

<213> Cochliobolus heterostrophus

5<400> 198

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Asp Val Glu Trp Glu Arg Cys Glu Ala Val Tyr Lys Tyr Thr Thr Gly
      20              25              30
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      35              40              45
Gln Arg Ser Ala Phe Thr Gly Ala Tyr Ile Met Arg Ile Glu Arg Gly
      50              55              60
Arg Arg Leu Arg Arg Ser Gln Ile Gln Thr Val Gln Gly Val Leu Ser
1565              70              75              80
Ser Leu Leu Ala His Ile Val Pro Ser Glu Leu Lys Leu Ser Met Pro
      85              90              95
Met Pro Cys Phe Arg Ser Ala Phe Ser Phe Phe Ser Arg Ala Ala Leu
      100              105              110
20Gly Gly Val Ala Gly Cys Gly Val Ser Val Lys Leu Arg Ala Pro Arg
      115              120              125
Leu Ser Thr Cys Ser Val Tyr Thr Gly Ala Leu Gly Thr Ser Met Thr
      130              135              140
Gly Val Trp Ser Arg Asn Arg Met Gly Ala Asn Val Arg Val Glu Arg
25145              150              155              160
Met Arg Thr Arg Arg Ser Gln Pro Pro Thr Ala Arg Arg Ser Cys Pro
      165              170              175
Gly Thr His Ser Arg Leu Leu Met Pro Arg Pro Ser Thr Ser His Ser
      180              185              190
30Leu Thr Ser Ser Asp Ala Ser Arg Val Met Val Arg Tyr Lys Cys Pro
      195              200              205
Ser Ala Val Tyr Ile Lys Ala Cys Asn Pro Gln Thr Gln Arg Pro Arg
      210              215              220
Trp Pro Ala Ser Ala Arg His Asp Ser Ile Ser Asp Gln Arg Arg Leu
35225              230              235              240

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<210> 199

<211> 1647

<212> DNA

40<213> Cochliobolus heterostrophus

131

<400> 199

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|--------------|------------|------------|------------|-------------|-------------|------|
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| gaagaaggcg | cttctgtgtg | tgaaagatgc | tctagcgtag | gcgtcgaatg | cattataccc | 120 |
| gagttccata | ttggtaggca | aaagggcgtg | aaaaacaaac | gatcaggggt | ggagaaagca | 180 |
| 5atctaccaag | tagaagaagc | aatcaagaag | agaaaatcag | acgtagctgt | caaccagagc | 240 |
| acgttacagc | atttgcaaca | gcttttgaac | gaagcacaag | gagacgttgg | ccctagtcaa | 300 |
| gatgcaaaat | caccgccagt | actagcagaa | ctatcttatg | tgccagcaaa | agaagttgcc | 360 |
| agcacttcaa | gcgatgatca | gcttgccgtt | gaagatgtcg | agaatccgct | tcagctttta | 420 |
| gcccgcgcac | cagacttgag | gattgccacc | acccacagct | cgtacaatac | aagtgtcgcc | 480 |
| 10agcccagaag | gcaggtttac | tggtagcgag | caaagcgcac | tcctcgatgt | tcataccttc | 540 |
| ttcttaccac | tgaaggcgca | tttggaacaa | ggatctgggt | tagatccaat | tgatgtagga | 600 |
| ttggttacca | aagatgaagc | ggagatgctc | ctccaatatt | tccacaaaag | actagctcac | 660 |
| acgcgctggg | gtctagaccc | agtggatgat | actctacctt | ttgtccgaaa | ccgctcagcc | 720 |
| tttctgttta | cgacattgct | ggctgtgacg | gccgtcttcc | taccagaaac | gtctgctttg | 780 |
| 15gccaaaagac | tacttcttca | ccgcagggtt | ctagctgaac | aggtcattgt | tcgaaagtac | 840 |
| agatccggtg | aaatcgtcct | ggcattcatg | gtgagcatac | catggatgcc | cccagggctc | 900 |
| catgcaagcg | acgacgacac | aagtctctat | ctagctacgg | cattgtctat | ttctttggat | 960 |
| cttatgctag | acaaagtcac | cactccatct | acgtcctttg | gtccggagct | cacgaggcag | 1020 |
| atgcccaaag | cagagtgtct | tgacgcaaga | aaagcactag | ctatggatgg | tttcgaggac | 1080 |
| 20attgacccga | cttctgaatg | gggccagcga | ctgcttcgtc | ggagagaaaag | ggtctggatt | 1140 |
| gcgctgtttg | tgctagagcg | tgccgtgtgc | ctcgctcggt | gccgcagcta | ctgtgtacca | 1200 |
| aagacgtgct | tgattcaata | cagcgataaa | tggcatgacc | accagcactc | ggatgcccag | 1260 |
| gacggctccg | tagtatccat | ggcagtatta | cgtcgcgatc | tcgacaacct | ttttgccgaa | 1320 |
| gtacgcacgc | gatgcgacaa | ctatggctcg | gccgaagtag | gttcccaggt | tgccgcaggaa | 1380 |
| 25atcgacaagt | caattgaggg | cttcttcgac | aattggtctc | gggcatggcc | ttcagttata | 1440 |
| agtgacccag | agagcaagag | cctacccctt | tatgtcgaga | tactcggttac | acacacacga | 1500 |
| ctctcgacct | actcaatgct | tctgaacctt | ccgagcgcgc | caccagaagt | caagcgctcg | 1560 |
| ttccgcaagt | ctgcgttatc | ctcggcgctc | aatgttatgc | gccgcagcaa | tccaaggcga | 1620 |
| gggacctctc | aagtcaatgc | ccaataa | | | | 1647 |

30

<210> 200

<211> 548

<212> PRT

<213> Cochliobolus heterostrophus

35

<400> 200

Met Asn Val Lys Gln Ala Ala Cys Leu Asn Cys Arg Lys Ser Lys Ile

1

5

10

15

Lys Cys Arg Arg Glu Glu Gly Ala Ser Val Cys Glu Arg Cys Ser Ser

40

20

25

30

132

Val Gly Val Glu Cys Ile Ile Pro Glu Phe His Ile Gly Arg Gln Lys
 35 40 45
 Gly Val Lys Asn Lys Arg Ser Gly Leu Glu Lys Ala Ile Tyr Gln Val
 50 55 60
 5Glu Glu Ala Ile Lys Lys Arg Lys Ser Asp Val Ala Val Asn Gln Ser
 65 70 75 80
 Thr Leu Gln His Leu Gln Gln Leu Leu Asn Glu Ala Gln Gly Asp Val
 85 90 95
 Gly Pro Ser Gln Asp Ala Lys Ser Pro Pro Val Leu Ala Glu Leu Ser
 10 100 105 110
 Tyr Val Pro Ala Lys Glu Val Ala Ser Thr Ser Ser Asp Asp Gln Leu
 115 120 125
 Ala Val Glu Asp Val Glu Asn Pro Leu Gln Leu Leu Ala Arg Ala Ser
 130 135 140
 15Asp Leu Arg Ile Ala Thr Thr Pro Gln Ser Tyr Asn Thr Ser Val Ala
 145 150 155 160
 Ser Pro Glu Gly Arg Phe Thr Gly Ser Glu Gln Ser Ala Phe Leu Asp
 165 170 175
 Val His His Phe Phe Leu Pro Met Lys Ala His Leu Asp Gln Gly Ser
 20 180 185 190
 Gly Leu Asp Pro Ile Asp Val Gly Leu Val Thr Lys Asp Glu Ala Glu
 195 200 205
 Met Leu Leu Gln Tyr Phe His Lys Arg Leu Ala His Thr Arg Trp Gly
 210 215 220
 25Leu Asp Pro Val Val His Thr Leu Pro Phe Val Arg Asn Arg Ser Ala
 225 230 235 240
 Phe Leu Phe Thr Thr Leu Leu Ala Val Thr Ala Val Phe Leu Pro Glu
 245 250 255
 Thr Ser Ala Leu Ala Lys Arg Leu Leu Leu His Arg Arg Phe Leu Ala
 30 260 265 270
 Glu Gln Val Ile Val Arg Lys Tyr Arg Ser Val Glu Ile Val Leu Ala
 275 280 285
 Phe Met Val Ser Ile Pro Trp Met Pro Pro Gly Ser His Ala Ser Asp
 290 295 300
 35Asp Asp Thr Ser Leu Tyr Leu Ala Thr Ala Leu Ser Ile Ser Leu Asp
 305 310 315 320
 Leu Met Leu Asp Lys Val Ile Thr Pro Ser Thr Ser Phe Gly Pro Glu
 325 330 335
 Leu Thr Arg Gln Met Pro Lys Ala Glu Cys Leu Asp Ala Arg Lys Ala
 40 340 345 350

133

Leu Ala Met Asp Gly Phe Glu Asp Ile Asp Pro Thr Ser Glu Trp Gly
 355 360 365
 Gln Arg Leu Leu Arg Arg Arg Glu Arg Val Trp Ile Ala Leu Phe Val
 370 375 380
 5Leu Glu Arg Gly Val Cys Leu Ala Arg Gly Arg Ser Tyr Cys Val Pro
 385 390 395 400
 Lys Thr Cys Leu Ile Gln Tyr Ser Asp Lys Trp His Asp His Gln His
 405 410 415
 Ser Asp Ala Gln Asp Gly Pro Leu Val Ser Met Ala Val Leu Arg Arg
 10 420 425 430
 Asp Leu Asp Asn Leu Phe Ala Glu Val Arg Thr Arg Cys Asp Asn Tyr
 435 440 445
 Gly Ser Ala Glu Val Gly Ser Gln Val Ala Gln Glu Ile Asp Lys Ser
 450 455 460
 15Ile Glu Gly Phe Phe Asp Asn Trp Ser Arg Ala Trp Pro Ser Val Ile
 465 470 475 480
 Ser Asp Pro Glu Ser Lys Ser Leu Pro Pro Tyr Val Glu Ile Leu Val
 485 490 495
 Thr His Thr Arg Leu Ser Thr Tyr Ser Met Leu Leu Asn His Pro Ser
 20 500 505 510
 Ala Pro Pro Glu Val Lys Arg Ser Phe Arg Lys Ser Ala Leu Ser Ser
 515 520 525
 Ala Leu Asn Val Met Arg Arg Ser Asn Pro Arg Arg Gly Thr Ser Gln
 530 535 540
 25Val Asn Ala Gln
 545

<210> 201

<211> 2271

30<212> DNA

<213> Cochliobolus heterostrophus

<400> 201

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| 35ggcggcatca | cctcgcggg | cctaccgct | tctatcacgg | tcaaagagct | ccgcaccgcg | 120 |
| atacacgatg | ctgtgcctc | caagcctgcc | cccgagcgca | tgcgctcat | atacagaggc | 180 |
| cgagtggtag | cgaatgatgc | agacactctg | actaccgtgt | ttggcgctga | caatatacgt | 240 |
| gagaacaaga | accaaagcct | tcacctcgtc | atacgagagc | tgctccaac | tgcatcttcg | 300 |
| cctgtcccgc | aatcgtcttc | tgtccacca | aacctcttcc | gctctgctgg | tccagatggc | 360 |
| 40ccagccgcga | gccctctgca | gacgaatcca | tttcgggcta | taccacagac | acgaccggct | 420 |

134

```

tcacaacctc aaatacccca gtcgcacctt ccgcctcatc gccttccggg acaagtgaac 480
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caaggacaac aggggtgatga acagccctca gatcgaacta gcgagcagcc agatcaaggt 600
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5ggaaatggcg accaggcgat caggcgagaa ggtggtgctc ctaatggagc acgatggaca 720
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30gctagtgaag ccaggggtga gatggacagt gaggttactc caagcagcag ttcaaagggc 2220
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<210> 202

<211> 756

35<212> PRT

<213> Cochliobolus heterostrophus

<400> 202

Met Ala Asp Ala Glu Gln Thr Ile Asn Leu Lys Val Leu Ser Pro Ser

40 1

5

10

15

135

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 20 25 30
 Thr Val Lys Glu Leu Arg Thr Arg Ile His Asp Ala Val Pro Ser Lys
 35 40 45
 5Pro Ala Pro Glu Arg Met Arg Leu Ile Tyr Arg Gly Arg Val Val Ala
 50 55 60
 Asn Asp Ala Asp Thr Leu Thr Thr Val Phe Gly Ala Asp Asn Ile Arg
 65 70 75 80
 Glu Asn Lys Asn Gln Ser Leu His Leu Val Ile Arg Glu Leu Pro Pro
 10 85 90 95
 Thr Ala Ser Ser Pro Val Pro Gln Ser Ser Ser Val Pro Pro Asn Leu
 100 105 110
 Phe Arg Ser Ala Gly Pro Asp Gly Pro Ala Ala Ser Pro Leu Gln Thr
 115 120 125
 15Asn Pro Phe Arg Ala Ile Pro Gln Thr Arg Pro Ala Ser Gln Pro Gln
 130 135 140
 Ile Pro Gln Ser His Leu Pro Pro His Arg Leu Pro Gly Gln Val Asn
 145 150 155 160
 Pro Ile Pro Ile Pro Leu Pro Ala Gln Leu His Gln Thr Phe Ala Gln
 20 165 170 175
 Ala Met Ala His Gln Gly Gln Gln Gly Asp Glu Gln Pro Ser Asp Arg
 180 185 190
 Thr Ser Glu Gln Pro Asp Gln Gly Thr Pro Ala Ala Gly Asp Arg Thr
 195 200 205
 25His Thr Pro Ile Pro Ser Gly Pro Ser Asn Pro Pro Gly Asn Gly Asp
 210 215 220
 Gln Ala Ile Arg Arg Glu Gly Val Ala Pro Asn Gly Ala Arg Trp Thr
 225 230 235 240
 Val Thr Ala Phe Asn Pro Leu Asn Ile Ala Ala Arg Leu Pro Pro Pro
 30 245 250 255
 Val Val Thr Phe Pro Val Pro His Ala Leu Thr Phe Gly Arg Pro Pro
 260 265 270
 Leu Ser Ser Asp Asn Gln Arg Leu Leu Pro Arg Val His Arg Ile Phe
 275 280 285
 35Leu Glu Thr Lys Arg Glu Ile Asp Asn Ile Arg Ala Leu Leu Gln Leu
 290 295 300
 Pro Gly Ala Ser Asp Ala Gln Ser Gly Gly Leu Leu Thr Ser Asp Ile
 305 310 315 320
 Pro Ala Ser Leu Asn Ile Pro Val Trp Arg Ile Glu Arg Leu Arg Gln
 40 325 330 335

136

His Leu Asn Thr Val Asn Gln Asn Leu Asp Val Val Asp Arg Ala Leu
 340 345 350
 Ala Leu Leu Pro Thr Glu Pro Glu Val Thr Ala Leu Arg Arg Ser Ala
 355 360 365
 5Thr Glu Leu Arg Val Asp Ala Ala Glu Leu Ser Ile Val Leu Asp Arg
 370 375 380
 Gln Gln Gly Glu Thr Ala Arg Ala Thr Ser Asp Thr Ala Pro Gly Val
 385 390 395 400
 Pro Thr Ile Ala Ala Ala Ser Ser Thr Thr Ser Gln Thr Arg Pro Gly
 10 405 410 415
 Asp Val Thr Gln Thr Val Pro Thr Asp Ala Pro Ala Glu Leu Phe Leu
 420 425 430
 Leu Ser Ser Pro Gln Gly Pro Val Gly Val Leu Phe Asp Gln Arg Gly
 435 440 445
 15Thr Tyr Thr Thr Ala Pro Met Val Pro Thr Leu Pro Phe Gln Ser Phe
 450 455 460
 Ser Ser Gln Phe Ala Gln Asn Arg Gln Leu Ile Ala Gly Leu Gly Gln
 465 470 475 480
 Gln Met Ala Gln Gly Thr Asn His Leu His Asn Gln Val Ser Asn Met
 20 485 490 495
 Gln Pro Thr Pro Ile Gly Gln Pro Val Ala Val Gly Gln Ala Gln Asp
 500 505 510
 His Asn Arg Gly Tyr Asp Gln Asn Gln Asn Gln Asn Gln Asn Gln Asn
 515 520 525
 25Gln Asn Gln Asn Asp Asn Gln Asn Gly Val Gln Pro Glu Glu Asn Asp
 530 535 540
 Arg Met Ala Asn Ile Ala Gly His Leu Trp Leu Ile Phe Lys Leu Ala
 545 550 555 560
 Val Phe Val Tyr Val Phe Ala Gly Gly Gly Gly Ile Tyr Arg Pro Val
 30 565 570 575
 Met Leu Gly Ala Ile Ala Gly Ile Val Tyr Leu Ala Gln Ile Gly Met
 580 585 590
 Phe Glu Asp Gln Ile Asn Tyr Val Arg Arg His Phe Glu Ala Leu Leu
 595 600 605
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 610 615 620
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 40 645 650 655

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Val Glu Arg Ala Phe Thr Leu Phe Ile Ala Ser Leu Phe Pro Gly Val
660 665 670
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675 680 685
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690 695 700
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138

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|----|---|--|-----|--|-----|
| | 20 | | 25 | | 30 |
| | Cys His Cys Glu Ser Pro His Leu Asp Val Ala Glu Lys Thr Thr Val | | | | |
| | 35 | | 40 | | 45 |
| | Glu Ile Ala Asn Ala Cys Ile Ile Thr Trp Arg Leu Pro Val Arg Ala | | | | |
| 5 | 50 | | 55 | | 60 |
| | Ser Ser Phe Glu Leu Leu Ser Asp Arg Asp Ala Ile Tyr Pro His Thr | | | | |
| | 65 | | 70 | | 75 |
| | His Met Arg Ala Ser Leu Pro His Leu Ala Pro Ser Arg Arg Leu Glu | | | | |
| | 85 | | 90 | | 95 |
| 10 | Arg His Ile Ala Asn Lys Ile Phe Thr Gly Arg Ser Leu Leu Gly Arg | | | | |
| | 100 | | 105 | | 110 |
| | Gly Ser Ile Pro His Ser Arg Ile Pro Lys Ser Asn Gly Ser Gln Tyr | | | | |
| | 115 | | 120 | | 125 |
| | Gln Leu His Ser Val Leu Ala Ser Trp Val His Phe Ser Tyr Ile Ala | | | | |
| 15 | 130 | | 135 | | 140 |
| | Tyr Asn Ala Glu Lys Ser Ile Gln Lys Pro Arg Glu Gly Met Arg Met | | | | |
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| cgatgcaata | taccacacaca | cccacatgcg | cgctctctcg | ccccatctcg | caccttcacg | 120 |
| tcgactcgag | cgtcatatag | cgaacaaaat | tttcacagga | aggagtcttt | taggtcgcg | 180 |
| 35ctcaattccg | cactcaagaa | taccaaagtc | aaatgggagc | caataccaat | tgactcgggt | 240 |
| attggcttcc | tgggtgcatt | tcagctatat | cgcatacaac | gcagagaaaa | gcatacagaa | 300 |
| gccgagagaa | gggatgcgga | tggcaatgta | gtggatcagc | aaggctgtcc | gaagaagcgc | 360 |
| gaaagaataa | gaccgagcgg | accatggacc | gttcagggtca | tgtctaccct | tcctctcaag | 420 |
| gcgttgctcg | gactgtgggg | tcgcttcaat | gagatcgaca | taccctacta | ccttcactta | 480 |
| 40catgtatacc | ccaacctcgc | cgcctttttc | taccgcaccc | tcaaaccgcg | tgtacgtcct | 540 |

139

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ctaggatcta caaggncag tacaccagag caaaatgtag caaattccca aattcgcgct      720
agtgagcacg agaagacacc acaagacgaa gaggacactg tgcgcgcgga tgaggaattt      780
5gcaaacgtga acggtatctc atatactcta ccaaactct tctccggacc atggccaaaa      840
gacgggaagc ctgctgaaat gccgacggat caatcagttc cgtcaaagcc atcgtcagaa      900
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tcattaaaga cacctacggt tctctactac tgcgttgat atcttgcgcc aggcgactac     1020
cacaggttcc actcacctgt atcatgggtt gttgagtcgc gtcgtaactt tgctggcgag     1080
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15cgcgggcgagg aaatgggtgg ttttcagttg ggcagtacaa ttgtcttagt ctttgaagcg     1440
ccgaagggca ttcgacctag tttggacgag ggcttttagtg gtacacgtgg cgagagaaaa     1500
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<221> SITE

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35Ser Ala Pro Ser Arg Thr Phe Thr Ser Thr Arg Ala Ser Tyr Ser Glu
          35              40              45
Gln Asn Phe His Arg Lys Glu Ser Phe Arg Ser Arg Leu Asn Ser Ala
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Leu Lys Asn Thr Lys Val Lys Trp Glu Pro Ile Pro Ile Ala Leu Gly
4065              70              75              80

```

| | | | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| Ile | Gly | Phe | Leu | Gly | Ala | Phe | Gln | Leu | Tyr | Arg | Ile | Gln | Arg | Arg | Glu | | |
| | | | | 85 | | | | | | | 90 | | | 95 | | | |
| Lys | His | Thr | Glu | Ala | Glu | Arg | Arg | Asp | Ala | Asp | Gly | Asn | Val | Val | Asp | | |
| | | | | 100 | | | | | | | 105 | | | 110 | | | |
| 5Gln | Gln | Gly | Arg | Pro | Lys | Lys | Arg | Glu | Arg | Ile | Arg | Pro | Ser | Gly | Pro | | |
| | | | | 115 | | | | | | | 120 | | | 125 | | | |
| Trp | Thr | Val | Gln | Val | Met | Ser | Thr | Leu | Pro | Leu | Lys | Ala | Leu | Ser | Arg | | |
| | | | | 130 | | | | | | | 135 | | | 140 | | | |
| Leu | Trp | Gly | Arg | Phe | Asn | Glu | Ile | Asp | Ile | Pro | Tyr | Tyr | Leu | His | Leu | | |
| 10145 | | | | | 150 | | | | | | | 155 | | | 160 | | |
| His | Val | Tyr | Pro | Asn | Leu | Ala | Ala | Phe | Phe | Tyr | Arg | Thr | Leu | Lys | Pro | | |
| | | | | 165 | | | | | | | 170 | | | 175 | | | |
| Gly | Val | Arg | Pro | Leu | Asp | Pro | Asn | Pro | Asn | Ala | Val | Leu | Ser | Pro | Ala | | |
| | | | | 180 | | | | | | | 185 | | | 190 | | | |
| 15Asp | Gly | Lys | Ile | Ile | Gln | Phe | Gly | Thr | Ile | Glu | His | Gly | Glu | Val | Glu | | |
| | | | | 195 | | | | | | | 200 | | | 205 | | | |
| Gln | Val | Lys | Gly | Val | Thr | Tyr | Ser | Leu | Asp | Ala | Leu | Leu | Gly | Ser | Thr | | |
| | | | | 210 | | | | | | | 215 | | | 220 | | | |
| Arg | Xaa | Ser | Thr | Pro | Glu | Gln | Asn | Val | Ala | Asn | Ser | Gln | Ile | Arg | Ala | | |
| 20225 | | | | | 230 | | | | | | | 235 | | | 240 | | |
| Ser | Glu | His | Glu | Lys | Thr | Pro | Gln | Asp | Glu | Glu | Asp | Thr | Val | Arg | Ala | | |
| | | | | 245 | | | | | | | 250 | | | 255 | | | |
| Asp | Glu | Glu | Phe | Ala | Asn | Val | Asn | Gly | Ile | Ser | Tyr | Thr | Leu | Pro | Asn | | |
| | | | | 260 | | | | | | | 265 | | | 270 | | | |
| 25Leu | Phe | Ser | Gly | Pro | Trp | Pro | Lys | Asp | Gly | Lys | Pro | Ala | Glu | Met | Pro | | |
| | | | | 275 | | | | | | | 280 | | | 285 | | | |
| Thr | Asp | Gln | Ser | Val | Pro | Ser | Lys | Pro | Ser | Ser | Glu | Ala | Glu | Val | Arg | | |
| | | | | 290 | | | | | | | 295 | | | 300 | | | |
| Ala | Asp | Leu | Ala | Leu | Ser | Glu | Ser | Gln | Arg | Pro | Trp | Trp | Ala | Pro | Ala | | |
| 30305 | | | | | 310 | | | | | | | 315 | | | 320 | | |
| Ser | Leu | Lys | Thr | Pro | Thr | Val | Leu | Tyr | Tyr | Cys | Val | Val | Tyr | Leu | Ala | | |
| | | | | 325 | | | | | | | 330 | | | 335 | | | |
| Pro | Gly | Asp | Tyr | His | Arg | Phe | His | Ser | Pro | Val | Ser | Trp | Val | Val | Glu | | |
| | | | | 340 | | | | | | | 345 | | | 350 | | | |
| 35Ser | Arg | Arg | His | Phe | Ala | Gly | Glu | Leu | Tyr | Ser | Val | Ser | Pro | Tyr | Leu | | |
| | | | | 355 | | | | | | | 360 | | | 365 | | | |
| Gln | Arg | Thr | Met | Pro | Gly | Leu | Phe | Thr | Leu | Asn | Glu | Arg | Val | Val | Leu | | |
| | | | | 370 | | | | | | | 375 | | | 380 | | | |
| Leu | Gly | Arg | Trp | Arg | Trp | Gly | Phe | Phe | Ser | Tyr | Thr | Pro | Val | Gly | Ala | | |
| 40385 | | | | | 390 | | | | | | | 395 | | | 400 | | |

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142

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|----|---|--|-----|--|-----|
| | 20 | | 25 | | 30 |
| | Ala Cys Val Leu Arg Gln Lys Gly Cys Arg Asp Arg Asp Val Ile Leu | | | | |
| | 35 | | 40 | | 45 |
| | Pro Trp Ile Leu Ala Asp Arg Pro Gly His Pro Ser Ala Gly Gly His | | | | |
| 5 | 50 | | 55 | | 60 |
| | Ala Ile Tyr Arg Cys Ile Glu Ser Ser Thr Ser Leu Val His Ser Ser | | | | |
| | 65 | | 70 | | 75 |
| | Cys Gly His Glu Arg Gly Thr Arg His Ala Leu Ala Gln Thr Ala Gln | | | | |
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| 10 | Ser Arg Pro Phe Leu Ser Asp Glu Ala Val Ala Gly Pro Ile Gln Lys | | | | |
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| ctccgtcccc | gctcgatcct | cataatgtac | gctcctgtga | aagcactcct | ctggcacccc | 120 |
| tgcgacccca | atcgtctcgt | tatccaaact | gcgcatgacg | aaccagtcgt | gtacttgtac | 180 |
| acggcttcac | agcgctccca | ctcgacgtca | tcttcacatcg | cgaattcatc | ggccactacg | 240 |
| 25caacatccac | cgtccatcct | ctcgttatcc | cctcacattg | ctaaaccgcg | agtcgcgact | 300 |
| cccgcacgct | ggaccgtatc | ctggctctcg | ggccctgcag | ctagtagtaa | aaaaccttgt | 360 |
| ttcgcgctcg | cgcataactca | agcttccggt | gtcgtttggc | cggagggcaa | agaccagatt | 420 |
| ctgcggtttg | atcatgaaga | cgaagaagag | ggtgaagagg | agggcggaaga | ggagggcgag | 480 |
| gaagcgggat | cagatgatag | tttgatatgat | atactgactg | gccggacacc | ggtaccgggt | 540 |
| 30acaagagata | gcatggagga | aggggggttt | ggggatagta | caggcacagt | gcaggagcta | 600 |
| gatgatacgt | ttcgatcgcg | gcgacatgca | catcaagaac | atggaggaca | tgaggaacac | 660 |
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<212> PRT

<213> Cochliobolus heterostrophus

<400> 210

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143

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| | 20 | 25 | 30 |
| Val Lys Ala Leu Leu Trp His Pro Cys Asp Pro Asn Arg Leu Val Ile | | | |
| 5 | 35 | 40 | 45 |
| Gln Thr Ala His Asp Glu Pro Val Val Tyr Leu Tyr Thr Ala Ser Gln | | | |
| | 50 | 55 | 60 |
| Arg Ser His Ser Thr Ser Ser Ser Ser Ala Asn Ser Ser Ala Thr Thr | | | |
| 65 | 70 | 75 | 80 |
| 10Gln His Pro Pro Ser Ile Leu Ser Leu Ser Pro His Ile Ala Lys Pro | | | |
| | 85 | 90 | 95 |
| Ala Val Ala Thr Pro Ala Arg Trp Thr Val Ser Trp Leu Ser Gly Pro | | | |
| | 100 | 105 | 110 |
| Ala Ala Ser Ser Lys Lys Pro Cys Phe Ala Leu Ala His Thr Gln Ala | | | |
| 15 | 115 | 120 | 125 |
| Ser Val Val Val Trp Pro Glu Gly Lys Asp Gln Ile Leu Arg Phe Asp | | | |
| | 130 | 135 | 140 |
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| 145 | 150 | 155 | 160 |
| 20Glu Ala Gly Ser Asp Asp Ser Leu Tyr Asp Ile Leu Thr Gly Arg Thr | | | |
| | 165 | 170 | 175 |
| Pro Val Pro Gly Thr Arg Asp Ser Met Glu Glu Gly Gly Phe Gly Asp | | | |
| | 180 | 185 | 190 |
| Ser Thr Gly Thr Val Gln Glu Leu Asp Asp Thr Phe Arg Ser Arg Arg | | | |
| 25 | 195 | 200 | 205 |
| His Ala His Gln Glu His Gly Gly His Glu Glu His Glu Tyr Phe Glu | | | |
| | 210 | 215 | 220 |
| Glu Gly Val Leu Gly Asp Ser Gly Met Ser Glu Met Phe | | | |
| 225 | 230 | 235 | |

30

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
30 May 2002 (30.05.2002)

PCT

(10) International Publication Number
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15/53, 15/31, 1/21, 15/82, 9/02, 9/00, C07K 14/37, C12Q
1/18, G01N 33/573, 33/68, A61K 35/00, A01N 61/00,
A01H 5/00

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21 November 2001 (21.11.2001)

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(26) Publication Language: English

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60/252,732 22 November 2000 (22.11.2000) US

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(71) Applicants and

(72) Inventors: **YODER, Olen** [US/US]; 4939 Concannon Court, San Diego, CA 91230 (US). **TURGEON, Barbara, G.** [CA/US]; 4939 Concannon Court, San Diego, CA 92130 (US). **LU, Shen-wen** [US/US]; 604 Winston Court, Apt. 4, Ithaca, NY 14850 (US).

(74) Agents: **KERNER, Ann-Louise** et al.; Hale and Dorr LLP, 60 State Street, Boston, MA 02109 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:
31 December 2003

(15) Information about Correction:

Previous Correction:

see PCT Gazette No. 14/2003 of 3 April 2003, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FUNGAL GENE CLUSTER ASSOCIATED WITH PATHOGENESIS

(57) Abstract: Methods to identify orthologs ofungal CPS1 genes as well as fungal iron reductase and permease/and or MFS transporter genes, and uses thereof are provided.



WO 2002/042444 A3

INTERNATIONAL SEARCH REPORT

Inter I Application No
PCT/US 01/43381

A. CLASSIFICATION OF SUBJECT MATTER

| | | | | | |
|-------|-----------|-----------|-----------|----------|------------|
| IPC 7 | C12N15/52 | C12N15/53 | C12N15/31 | C12N1/21 | C12N15/82 |
| | C12N9/02 | C12N9/00 | C07K14/37 | C12Q1/18 | G01N33/573 |
| | G01N33/68 | A61K35/00 | A01N61/00 | A01H5/00 | |

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|---|
| P, X | <p>DATABASE EMBL 'Online! 25 January 2001 (2001-01-25) LU S.W.ET AL: "Cochliobolus heterostrophus peptide synthetase-like protein (CPS1) gene, complete cds" retrieved from EMBL Database accession no. AF332878 XP002230678 the whole document</p> <p style="text-align: center;">---</p> | <p>1-18, 21, 27-52, 54, 56-62</p> |
| A | <p>TURGAY K ET AL: "A GENERAL APPROACH FOR IDENTIFYING AND CLONING PEPTIDE SYNTHETASE GENES" PEPTIDE RESEARCH, NATICK, MA, US, vol. 7, no. 5, September 1994 (1994-09), pages 238-241, XP000978191 ISSN: 1040-5704</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p> | |

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

° Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

14 February 2003

Date of mailing of the international search report

05.06.03

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Turri, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/43381

G.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | <p>NIKOLSKAYA A N ET AL: "Identification of peptide synthetase-encoding genes from filamentous fungi producing host-selective phytotoxins or analogs" GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 165, no. 2, 20 November 1995 (1995-11-20), pages 207-211, XP004043143 ISSN: 0378-1119</p> <p>----</p> | |
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| T | <p>LU S.W. ET AL: "A gene cluster from the corn Cochliobolus heterostrophus required for nonribosomal peptide biosynthesis and general virulence of fungi" SECONDARY METABOLISM AND PATHOGENICITY ABSTRACTS, ABSTRACTS NUMBERS 197-287, 'Online! 14 December 2000 (2000-12-14), XP002230676 Retrieved from the Internet: <URL:http://www.fgsc.net/asilo99/posterabs5.htm> 'retrieved on 2003-02-10! cited in the application Abstracts of the Twentieth Fungal Genetics Conference, held March 23-28, 1999, Pacific Grove, California. See Abstract no. 245</p> <p>----</p> <p style="text-align: center;">-/--</p> | |

INTERNATIONAL SEARCH REPORT

Inter .l Application No

PCT/US 01/43381

C/(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| T | <p>LU S -W ET AL: "Cochliobolus heterostrophus and Fusarium graminearum: Evidence for a common virulence factor." PHYTOPATHOLOGY, vol. 91, no. 6 Supplement, June 2001 (2001-06), page S56 XP001122359 Joint Meeting of the American Phytopathological Society, the Mycological Society of America, and the Society of Nematologists; Salt Lake City, Utah, USA; August 25-29, 2001 ISSN: 0031-949X</p> <p>----</p> | |
| A | <p>STACHELHAUS ET AL: "Modular structure of peptide synthetases" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 270, no. 11, 17 March 1995 (1995-03-17), pages 6163-6169, XP002113293 ISSN: 0021-9258</p> <p>----</p> | |
| A | <p>STACHELHAUS AND M A MARAHIEL T: "Modular structure of genes encoding multifunctional peptide synthetases required for non-ribosomal peptide synthesis" FEMS MICROBIOLOGY LETTERS, AMSTERDAM, NL, vol. 125, 1995, pages 3-14, XP002094834 ISSN: 0378-1097</p> <p>----</p> | |
| A | <p>TURGAY K ET AL: "FOUR HOMOLOGOUS DOMAINS IN THE PRIMARY STRUCTURE OF GRSB ARE RELATED TO DOMAINS IN A SUPERFAMILY OF ADENYLATE-FORMING ENZYMES" MOLECULAR MICROBIOLOGY, BLACKWELL SCIENTIFIC, OXFORD, GB, vol. 6, no. 4, 1992, pages 529-546, XP001055896 ISSN: 0950-382X</p> <p>----</p> | |
| A | <p>YODER O C ET AL: "Fungal genomics and pathogenicity." CURRENT OPINION IN PLANT BIOLOGY, vol. 4, no. 4, August 2001 (2001-08), pages 315-321, XP002230677 ISSN: 1369-5266</p> <p>-----</p> | |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/43381

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 53, 55
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 19, 20, 22-26
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4 (all partially), 5, 6, 7 (partially), 8-18, 21, 27 (partially)-52, 54, 56-62

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-4 (all partially), 5, 6, 7 (partially), 8-18, 21, 27 (partially)-52, 54, 56-62

Isolated polynucleotides comprising the open reading frame of SEQ ID NO:46 and encoding a polypeptide having at least 80% identity to SEQ ID NO:47.

Isolated polypeptides; expression cassettes and vectors comprising said polynucleotides. Host cells.

Methods for identifying inhibitors of the polypeptide and for identifying agents that alter the phenotype of a fungal pathogen.

Isolated antibodies.

Therapeutic methods.

Transformed plants.

2. Claims: 1-4 (all partially), 5, 6, 7 (partially), 8-18, 21, 27 (partially)-52, 54, 56-62

As group 1, but with SEQ ID NOs:48 and 49

3. Claims: 1-4 (all partially), 5, 6, 7 (partially), 8-18, 21, 27 (partially)-52, 54, 56-62

As group 1, but with SEQ ID NOs:55 and 56

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 48-51, and 56-59 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 53, 55

Rule 39.1(v) PCT - Presentation of information

Continuation of Box I.2

Claims Nos.: 19, 20, 22-26

Present claims 19 and 25 relate to an extremely large number of possible compounds binding to the CPS1 polypeptide. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for none of the compounds. In the present case, the claim so lack support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible.

Present claims 20, 22 and 23 relate to an agent defined in terms of the method for identifying it. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is not to be found, however, for such agent. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible.

Present claims 24 and 26 relate to a compound defined by reference to a desirable characteristic or property, namely inhibiting the activity of the CPS1 polypeptide.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for none of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

CORRECTED VERSION

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60/252,732 22 November 2000 (22.11.2000) US(71) Applicants (for all designated States except US): **SYNGENTA PARTICIPATIONS AG** [CH/CH]; Schwarzwaldallee 215, CH-4058 (CH). **CORNELL RESEARCH FOUNDATION, INC.** [US/US]; 20 Fomwood Drive, Suite 105, Ithaca, NY 14850 (US).

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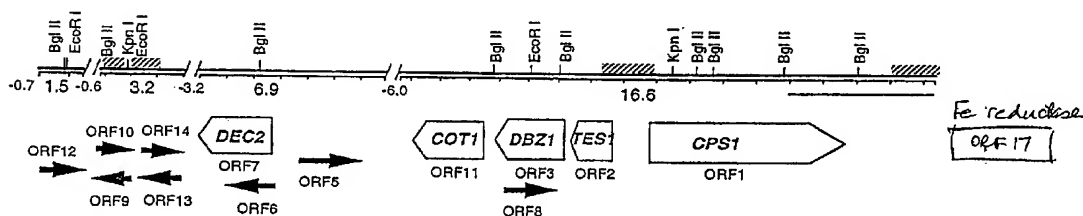
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FUNGAL GENE CLUSTER ASSOCIATED WITH PATHOGENESIS



ORF 15 permease, MFS transporter
ORF 16 lactase precursor

(57) Abstract: Methods to identify orthologs of ungal CPS1 genes as well as fungal iron reductase and permease/and or MFS transporter genes, and uses thereof are provided.

WO 02/042444 A2